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By: Helene Isabel Date: March 5, 2004

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Patent Application of: Christine Knox *et al.*			
Appln. No.:	10/715,926	Examiner:	Not Yet Assigned
Filing Date:	November 18, 2003	Attorney Docket No.:	10338-17US
Title:	ADHERENT ENTITIES AND USES THEREFOR		

CLAIM OF FOREIGN PRIORITY AND TRANSMITTAL OF PRIORITY DOCUMENT

Applicants hereby claim the right of foreign priority under 35 U.S.C. Section 119 for the above-identified patent application. The claim of foreign priority is based upon Application No. PR 5124, filed in Australia on May 18, 2001, and the benefit of that date is claimed.

Submitted herewith is a certified copy of Australian Application No. PR 5124. It is submitted that this document completes the requirements of 35 U.S.C. Section 119, and benefit of the foreign priority is respectfully requested.

Respectfully submitted,

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March 5, 2004 By: Alan S. Nadel
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I, JULIE BILLINGSLEY, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PR 5124 for a patent by QUEENSLAND UNIVERSITY OF TECHNOLOGY as filed on 18 May 2001.

WITNESS my hand this
Second day of March 2004

J. Billingsley

JULIE BILLINGSLEY
TEAM LEADER EXAMINATION
SUPPORT AND SALES

Regulation 3.2

A U S T R A L I A

Patents Act 1990

PROVISIONAL SPECIFICATION

for the invention entitled:

"Adherent entities and uses therefor"

The invention is described in the following statement:

ADHERENT ENTITIES AND USES THEREFOR

FIELD OF THE INVENTION

THIS INVENTION relates generally to pathogenic agents associated with genital tract infections. More particularly, the present invention relates to organisms of the genus *Ureaplasma*, especially strains or serotypes of the species *Ureaplasma parvum* and *U. urealyticum*, which adhere more strongly to spermatozoa than other strains or serotypes of *Ureaplasma*. Even more particularly, the invention relates to the detection of these organisms and to various therapeutic and prophylactic strategies for enhancing or otherwise improving *inter alia* fertility, especially male fertility. The invention also extends to an adhesin present on the surface of these organisms, to methods for its preparation and to adhesin-encoding polynucleotides. The present invention also extends to the development of methods for screening of agents useful *inter alia* for modulating an adhesin-encoding gene or for modulating the level and/or functional activity of an expression product of that gene. The invention also concerns the use of adhesin-containing compositions in the production of antigen binding molecules that are immuno-interactive with the adhesin. The invention further relates to the use of the adhesin, or a biologically-active fragment thereof, or a variant or derivative of these, and to the use of above modulatory agents in the preparation of compositions for the treatment and/or prophylaxis of infections caused by the aforesaid organisms, for the treatment of spermatozoa and for improving spermatozoal fertilisation of oocytes.

Bibliographic details of various publications referred to in this specification are collected at the end of the description.

BACKGROUND OF THE INVENTION

The ureaplasmas, the newly designated *U. parvum* (previously *U. urealyticum* serovars 1, 3, 6, and 14) *and U. urealyticum* (previously serovars 2, 4, 5, 7-13) are the microorganisms most frequently isolated from placental tissue with histological evidence of chorioamnionitis. This upper genital tract infection, frequently clinically asymptomatic, is significantly associated with adverse pregnancy sequelae including preterm delivery, premature onset of labour, prolonged rupture of membranes and neonatal morbidity and mortality (Cassell, *et al.*, 1993).

Ascending infection of the placenta occurs rapidly upon rupture of membranes but mid-trimester, persistent ureaplasma amnionitis has also been reported in the absence of premature labour or rupture of placental membranes (Cassell, *et al.*, 1986) (Cassell, *et al.*, 1983). In a previous study, the present inventors subtyped ureaplasma isolates from infected placental tissue and from the female lower genital tract and detected different ureaplasma subtypes in these sites (Knox and Timms, 1998). They have also shown that the male urogenital tract and the female lower genital tract may be separately colonised with different ureaplasma subtypes. These findings are consistent with an exogenous reservoir, the male urogenital tract, as the source of upper genital tract infection (of the placenta and the foetus) in pregnant women.

Several studies have investigated the effect of ureaplasma positive semen samples on ART outcomes. These studies found (i) reduced pregnancy rates per embryo transfer (Montagut, *et al.*, 1991; Shalika, *et al.*, 1996) (ii) reduced blastocyst culture rates (Riedel, *et al.*, 1986) and (iii) an increased miscarriage rate (Kanakas, *et al.*, 1999) when the male semen was infected or colonised with ureaplasmas.

In work leading up to the present invention, the inventors studied the effect of ureaplasma positive washed semen on: fertilisation by *in vitro* fertilisation (IVF) or by intracytoplasmic injection (ICSI); and on ART clinical outcomes, the viable pregnancy rate and the miscarriage rate. The effect of different ureaplasma subtypes on these outcomes was also examined. This study demonstrated a reduced viable pregnancy rate per embryo transfer (6.7%) in ICSI couples with ureaplasma positive washed semen, compared to couples with ureaplasma positive semen, negative washed semen (23%), and couples with ureaplasma negative semen and washed semen but ureaplasma positive endocervical swabs (10%) and ureaplasma negative couples (15%). This study also showed an increase in the miscarriage rate (miscarriages/no clinical pregnancies) for ureaplasma washed semen positive couples (56%) compared to couples with ureaplasma positive semen, negative washed semen (11 %), and couples with ureaplasma negative semen and washed semen but ureaplasma positive endocervical swabs (25%) and ureaplasma negative couples (35%).

Unexpectedly, this investigation also revealed that certain strains or serotypes of *Ureaplasma* adhere more strongly to spermatozoa than other strains or serotypes of this genus and, consequently, remain adherent to spermatozoa after washing. Taken together, these results indicate that these more strongly adherent members of *Ureaplasma* are more

likely to impact adversely on ART pregnancy outcomes, and strongly support the hypothesis that ureaplasma adherence to spermatozoa is a mechanism of pathogenicity facilitating infection of the embryo at conception. The aforementioned discoveries have been reduced to practice *inter alia* in methods for detecting strongly spermatozoal adherent strains or serotypes of *Ureaplasma,* in methods of diagnosis of conditions associated with these strains or serotypes and in various therapeutic and prophylactic strategies for enhancing or otherwise improving fertility, especially male fertility, as described hereinafter.

SUMMARY OF THE INVENTION

Accordingly, in one aspect of the present invention, there is provided a method for detecting an ureaplasma associated with a higher rate of male infertility, comprising separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm, whereby detection of said adherent ureaplasma after said separation indicates an association of said ureaplasma with a higher rate of male infertility.

Preferably, the separation conditions protect one or more viability properties of the sperm.

Suitably, separation of sperm from non-sperm substances is effected by a sperm swim-up technique.

Alternatively, said separation is effected by washing the sperm in a washing solution. In a preferred embodiment of this type, the sperm sample is washed using gradient centrifugation.

In a preferred embodiment, the ureaplasma is *Ureaplasma parvum* serotype 6.

In another aspect, the invention envisions a method for detecting an ureaplasma associated with a lower rate of oocyte fertilisation, comprising separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm, whereby detection of said adherent

ureaplasma after said separation indicates an association of said ureaplasma with a lower rate of oocyte fertilisation.

In yet another aspect, the invention resides in a method for detecting an ureaplasma associated with an adverse pregnancy outcome, comprising separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm, whereby detection of said adherent ureaplasma after said separation indicates an association of said ureaplasma with said adverse pregnancy outcome.

In still yet another aspect, the invention encompasses a method of detecting an ureaplasma associated with an adverse assisted reproductive technology (ART) outcome, comprising separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm, whereby detection of said adherent ureaplasma after said separation indicates an association of said ureaplasma with said adverse ART outcome.

In yet another aspect, the invention resides in a method of detecting an ureaplasma associated with a higher rate of male infertility, comprising detecting the presence of an ureaplasma that remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm.

In another aspect, the invention envisions a method of detecting an ureaplasma associated with a lower rate of oocyte fertilisation, comprising detecting the presence of an ureaplasma that remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm.

In yet another aspect, the invention resides in a method of detecting an ureaplasma associated with an adverse pregnancy outcome, comprising detecting the presence of an ureaplasma that remains adherent to sperm after separating sperm from non-sperm

substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm.

In a further aspect, the invention features a method of ascertaining the propensity for infertility in a male patient, comprising detecting an ureaplasma in a sperm sample obtained from the patient, wherein the sperm has been separated from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm, and wherein the presence of said adherent ureaplasma in the sample indicates that said patient has an increased propensity for infertility.

In yet a further aspect, the invention contemplates a method for diagnosis of a lower rate of oocyte fertilisation by sperm of a patient, comprising detecting an ureaplasma in a sperm sample obtained from the patient, wherein the sperm has been separated from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm, and wherein the presence of said adherent ureaplasma in the sample indicates that said sperm has a reduced rate of oocyte fertilisation.

Suitably, the fertilisation is effected by ART procedure. In a preferred embodiment, the ART procedure is selected from artificial insemination (AI), *in vitro* fertilisation (IVF) and intracytoplasmic sperm injection (ICSI). Alternatively, the fertilisation is effected by natural means.

In still yet a further aspect, the invention contemplates a method of ascertaining the propensity for an adverse pregnancy outcome in a patient, comprising detecting an ureaplasma in a sperm sample obtained from the patient's sperm donor, wherein the sperm has been separated from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm, and wherein the presence of said adherent ureaplasma in the sample indicates that said patient has an increased propensity for an adverse pregnancy outcome.

In yet another aspect, the invention encompasses a method of determining the propensity of a sperm sample for an adverse assisted reproductive technology (ART) outcome, comprising detecting the presence of an ureaplasma in said sperm sample, wherein the sperm has been separated from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm, and wherein the presence of said adherent ureaplasma in the sample indicates that said sperm has an increased propensity for an adverse ART outcome.

In yet another aspect, the invention features a method for diagnosis of a high risk of infertility in a male patient, comprising detecting an ureaplasma in a biological sample obtained from the patient, wherein said ureaplasma remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm, and wherein the presence of said adherent ureaplasma in the sample indicates that said patient has an increased propensity for infertility.

Suitably, the biological sample comprises a biological fluid selected from whole blood, serum, plasma, saliva, urine, sweat, ascitic fluid, peritoneal fluid, synovial fluid, and cerebrospinal fluid, amniotic fluid, seminiferous tubule fluid (*e.g.*, in vasectomised men), semen, vaginal secretions, endocervical secretions, respiratory secretions, endometrial lining, fallopian tube washings. Alternatively, the biological sample comprises a tissue biopsy including, but not restricted to, placental tissue, seminiferous tubules, ovarian tissue and fallopian tube tissue.

In yet a further aspect, the invention contemplates a method for diagnosis of a high risk of unsuccessful oocyte fertilisation by sperm of a patient, comprising detecting an ureaplasma in a biological sample obtained from the patient, wherein said ureaplasma remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm, and wherein the presence of said adherent ureaplasma in the sperm sample indicates that said sperm has a reduced rate of oocyte fertilisation.

In still yet a further aspect, the invention contemplates a method for diagnosis of a high risk of an adverse pregnancy outcome in a patient, comprising detecting an ureaplasma in a biological sample obtained from the patient's sperm donor, wherein said ureaplasma remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm, and wherein the presence of said adherent ureaplasma in the sample indicates that said patient has an increased propensity for an adverse pregnancy outcome.

In yet another aspect, the invention encompasses a method for diagnosis of a high risk of an adverse assisted reproductive technology (ART) outcome using sperm of a patient, comprising detecting an ureaplasma in a biological sample obtained from the patient, wherein said ureaplasma remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm, and wherein the presence of said adherent ureaplasma in the sample indicates that said sperm has an increased propensity for an adverse ART outcome.

In still yet a further aspect, the invention contemplates a method for diagnosis of a high risk of an adverse pregnancy outcome, or of an adverse assisted reproductive technology (ART) outcome, in a patient, comprising detecting an ureaplasma in a biological sample obtained from the patient, or from a donor of a procreation element selected from a spermatozoon, an oocyte or an embryo, wherein said ureaplasma remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm, and wherein the presence of said adherent ureaplasma in the sample indicates that said patient has an increased propensity for an adverse pregnancy outcome or for an adverse ART outcome.

The present inventors consider that the enhanced adherence to, or affinity for, spermatozoa displayed by certain ureaplasmas is mediated by one or more adhesins present on the surface of those ureaplasmas. Accordingly, in another aspect, the invention

contemplates an isolated ureaplasma adhesin, or a biologically active fragment thereof, or a variant or derivative of these, which ureaplasma remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm.

In another aspect, the invention encompasses an isolated polynucleotide encoding the adhesin, or the biologically active fragment thereof, or the variant or derivative of these, as broadly described above.

In yet another aspect of the present invention, there is provided a method for detecting an ureaplasma associated with a higher rate of male infertility, comprising detecting a gene encoding an ureaplasma adhesin or an expression product of said gene, wherein said ureaplasma remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm.

In still yet another aspect, the invention resides in a method for detecting an ureaplasma associated with a lower rate of oocyte fertilisation, comprising detecting a gene encoding an ureaplasma adhesin or an expression product of said gene, wherein said ureaplasma remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm.

In a further aspect, the invention encompasses a method for detecting an ureaplasma associated with a higher incidence of an adverse pregnancy outcome, comprising detecting a gene encoding an ureaplasma adhesin or an expression product of said gene, wherein said ureaplasma remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm.

In another aspect, the invention envisions a method for detecting an ureaplasma associated with a higher incidence of an adverse assisted reproductive technology (ART)

outcome, comprising detecting a gene encoding an ureaplasma adhesin or an expression product of said gene, wherein said ureaplasma remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm.

The present inventors consider that the ureaplasma adhesin as broadly described above, as well as the gene encoding that adhesin, can be used to provide both drug targets and regulators to promote or inhibit the adherence of said adhesin to spermatozoa and to provide diagnostic markers for male infertility, adverse pregnancy outcomes or adverse assisted reproductive technology (ART) outcomes, using, for example, detectable polypeptides and polynucleotides as broadly described above, or using detectable agents which interact specifically with those polypeptides or polynucleotides.

Thus, in another aspect, the invention extends to a method of screening for an agent which modulates the adherence of an ureaplasma to spermatozoa, wherein said ureaplasma remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm, said method comprising:

- contacting a preparation comprising a first member selected from the group consisting of an ureaplasma adhesin, a biologically active fragment of said adhesin, a variant of said adhesin, a variant of said biologically active fragment, a derivative of said adhesin, a derivative of said biologically active fragment, and a derivative of said variant, or a second member selected from the group consisting of a genetic sequence that regulates or encodes said first member and a fragment of said genetic sequence, with a test agent; and

- detecting a change in the level and/or functional activity of said first member or an expression product of said second member.

In another aspect, the invention resides in the use of an ureaplasma adhesin, or a biologically active fragment thereof, or a variant or derivative of these, in crude or substantially purified form, to produce an antigen-binding molecule that is immuno-interactive with said adhesin, said biologically active fragment, said variant or said

derivative, wherein said ureaplasma remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm.

In yet another aspect, the invention provides an antigen-binding molecule so produced.

According to another aspect of the invention, there is provided a method of detecting in a biological sample an ureaplasma adhesin, or a biologically active fragment thereof, or a variant or derivative of these, which ureaplasma remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm, said method comprising:

- contacting the sample with an antigen-binding molecule as broadly described above; and
- detecting the presence of a complex comprising said antigen-binding molecule and said adhesin, fragment, variant or derivative in said contacted sample.

In yet another aspect, the invention contemplates a method for prognostic assessment of male infertility, adverse pregnancy outcome or adverse assisted reproductive technology (ART) outcome, comprising detecting, in a biological sample obtained from a patient, expression of a gene encoding an ureaplasma adhesin, which ureaplasma remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm.

In one embodiment, the method comprises detecting the presence in said biological sample of a gene encoding said adhesin.

In another embodiment, the method comprises detecting the presence in said biological sample of an expression product of said gene.

In yet another aspect of the invention, there is provided a method for masking an ureaplasma adhesin or for otherwise interfering with the binding of said adhesin to sperm, wherein said ureaplasma remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm, said method comprising contacting said adhesin with an antigen-binding molecule that is immuno-interactive with said adhesin.

In another aspect of the invention, there is provided a method for modulating adherence of an ureaplasma to sperm, which ureaplasma remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm, said method comprising contacting said ureaplasma with an agent as broadly described above for a time and under conditions sufficient to modulate the level and/or functional activity of said adhesin.

The agent preferably decreases the level and/or functional activity of said adhesin.

In yet another aspect, the invention provides a composition for enhancing or otherwise improving male fertility, comprising an agent that reduces the level and/or functional activity of an ureaplasma adhesin, wherein said ureaplasma remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm, and optionally together with a pharmaceutically acceptable carrier.

In still yet another aspect, the invention provides a composition for enhancing the propensity for a favourable pregnancy outcome, comprising an agent that reduces the level and/or functional activity of an ureaplasma adhesin, wherein said ureaplasma remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm, and optionally together with a pharmaceutically acceptable carrier.

In another aspect, the invention extends to a composition for enhancing the propensity for a favourable assisted reproductive technology (ART) outcome, comprising an agent that reduces the level and/or functional activity of an ureaplasma adhesin, wherein said ureaplasma remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm, and optionally together with a pharmaceutically acceptable carrier.

According to another aspect of the invention, there is provided a method for treatment and/or prophylaxis of male infertility, comprising administering to a patient in need of such treatment a therapeutically effective amount of an agent that reduces the level and/or functional activity of an ureaplasma adhesin, wherein said ureaplasma remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm, and optionally together with a pharmaceutically acceptable carrier.

The invention, in yet another aspect, contemplates a method for treatment and/or prophylaxis of male infertility, comprising administering to the sperm of a patient in need of such treatment a therapeutically effective amount of an agent that reduces the level and/or functional activity of an ureaplasma adhesin, wherein said ureaplasma remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm, and optionally together with a pharmaceutically acceptable carrier.

In still yet another aspect, the invention provides a method for enhancing the propensity for a favourable pregnancy outcome in a patient, comprising administering to the patient's sperm donor a therapeutically effective amount of an agent that reduces the level and/or functional activity of an ureaplasma adhesin, wherein said ureaplasma remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm, and optionally together with a pharmaceutically acceptable carrier.

In another aspect, the invention encompasses a method for enhancing the propensity for a favourable pregnancy outcome in a patient, comprising administering to the sperm of the patient's sperm donor a therapeutically effective amount of an agent that reduces the level and/or functional activity of an ureaplasma adhesin, wherein said ureaplasma remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm, and optionally together with a pharmaceutically acceptable carrier.

In still yet another aspect, the invention extends to a method for enhancing the propensity for a favourable assisted reproductive technology (ART) outcome in a patient, comprising administering to the patient's sperm donor a therapeutically effective amount of an agent that reduces the level and/or functional activity of an ureaplasma adhesin, wherein said ureaplasma remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm, and optionally together with a pharmaceutically acceptable carrier.

In another aspect, the invention encompasses a method for enhancing the propensity for a favourable assisted reproductive technology (ART) outcome in a patient, comprising administering to the sperm of the patient's sperm donor a therapeutically effective amount of an agent that reduces the level and/or functional activity of an ureaplasma adhesin, wherein said ureaplasma remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm, and optionally together with a pharmaceutically acceptable carrier.

According to another aspect, the invention extends to an immunopotentiating composition for eliciting the production of elements that specifically bind to an ureaplasma that remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype

to said sperm, said composition comprising a proteinaceous molecule selected from the group consisting of an isolated adhesin of said ureaplasma, a biologically active fragment of said adhesin, a variant of said adhesin, a variant of said biologically active fragment, a derivative of said adhesin, a derivative of said biologically active fragment, and a derivative of said variant, and/or or a vector comprising a polynucleotide encoding said proteinaceous molecule, wherein said polynucleotide is operably linked to a regulatory polynucleotide, wherein said composition optionally further comprises one or more pharmaceutically acceptable carriers, adjuvants and/or diluents.

In yet another aspect, the invention encompasses a method for treatment and/or prophylaxis of male infertility, comprising administering to a patient in need of such treatment an immunogenically effective amount of the immunopotentiating composition as broadly described above.

In still yet another aspect, the invention features a method for treatment and/or prophylaxis of an adverse pregnancy outcome in a patient, comprising administering to the patient's sperm donor an immunogenically effective amount of the immunopotentiating composition as broadly described above.

In a further aspect, the invention envisions a method for treatment and/or prophylaxis of an adverse assisted reproductive technology (ART) outcome in a patient, comprising administering to the patient's sperm donor an immunogenically effective amount of the immunopotentiating composition as broadly described above.

The invention also encompasses the use of the adhesin, fragment, variant or derivative as well as the modulatory agents as broadly described above in the study, and modulation of male infertility, pregnancy or assisted reproductive technology (ART) outcome.

DETAILED DESCRIPTION OF THE INVENTION

1. Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are described. For the purposes of the present invention, the following terms are defined below.

The articles *"a"* and *"an"* are used herein to refer to one or to more than one (i.e. to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

By *"agent"* is meant a naturally occurring or synthetically produced molecule which interacts either directly or indirectly with a target member, the level and/or functional activity of which are to be modulated.

"Amplification product" refers to a nucleic acid product generated by nucleic acid amplification techniques.

By *"antigen-binding molecule"* is meant a molecule that has binding affinity for a target antigen. It will be understood that this term extends to immunoglobulins, immunoglobulin fragments and non-immunoglobulin derived protein frameworks that exhibit antigen-binding activity.

As used herein, the term *"binds specifically" "specifically immuno-interactive"* and the like refers to antigen-binding molecules that bind, or are otherwise immuno-interactive with, the polypeptide or polypeptide fragments of the invention but do not significantly bind to, or do not otherwise specifically immuno-interact with, homologous prior art polypeptides.

By *"biologically active fragment"* is meant a fragment of a full-length parent polypeptide which fragment retains the activity of the parent polypeptide. A biologically active fragment will therefore adhere to sperm, or elicit an immunogenic response to produce elements (*e.g.*, antigen-binding molecules) that specifically bind to the parent

polypeptide. As used herein, the term *"biologically active fragment"* includes deletion mutants and small peptides, for example of at least 8, preferably at least 10, more preferably at least 15, even more preferably at least 20 and even more preferably at least 30 contiguous amino acids, which comprise the above activities. . Peptides of this type may be obtained through the application of standard recombinant nucleic acid techniques or synthesised using conventional liquid or solid phase synthesis techniques. For example, reference may be made to solution synthesis or solid phase synthesis as described, for example, in Chapter 9 entitled *"Peptide Synthesis"* by Atherton and Shephard which is included in a publication entitled *"Synthetic Vaccines"* edited by Nicholson and published by Blackwell Scientific Publications. Alternatively, peptides can be produced by digestion of a polypeptide of the invention with proteinases such as endoLys-C, endoArg-C, endoGlu-C and staphylococcus V8-protease. The digested fragments can be purified by, for example, high performance liquid chromatographic (HPLC) techniques.

The term *"biological sample"* as used herein refers to a sample that may be extracted, untreated, treated, diluted or concentrated from an animal. The biological sample may be selected from the group consisting of whole blood, serum, plasma, saliva, urine, sweat, ascitic fluid, peritoneal fluid, synovial fluid, amniotic fluid, cerebrospinal fluid, skin biopsy, and the like. Preferably, the biological sample is selected from amniotic fluid, seminiferous tubule fluid (*e.g.*, in vasectomised men), semen, vaginal secretions, endocervical secretions, respiratory secretions, endometrial lining, fallopian tube washings, placental tissue, chorioamnion, seminiferous tubule tissue , ovarian tissue, fallopian tube tissue.

Throughout this specification, unless the context requires otherwise, the words *"comprise"*, *"comprises"* and *"comprising"* will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements.

By "*corresponds to*" or "*corresponding to*" is meant a polynucleotide (a) having a nucleotide sequence that is substantially identical or complementary to all or a portion of a reference polynucleotide sequence or (b) encoding an amino acid sequence identical to an amino acid sequence in a peptide or protein. This phrase also includes within its scope a peptide or polypeptide having an amino acid sequence that is substantially identical to a sequence of amino acids in a reference peptide or protein.

By *"derivative"* is meant a polypeptide that has been derived from the basic sequence by modification, for example by conjugation or complexing with other chemical moieties or by post-translational modification techniques as would be understood in the art. The term "*derivative*" also includes within its scope alterations that have been made to a parent sequence including additions, or deletions that provide for functionally equivalent molecules. Accordingly, the term derivative encompasses molecules that will adhere to sperm, and the elicitation of an immunogenic response to produce elements (*e.g.*, antigen-binding molecules) that specifically bind to the parent adhesin.

As used herein, the term *"function"* refers to a biological, enzymatic, or therapeutic function.

"Homology" refers to the percentage number of amino acids that are identical or constitute conservative substitutions as defined in TABLE A *infra*. Homology may be determined using sequence comparison programs such as GAP (Deveraux *et al.* 1984). In this way, sequences of a similar or substantially different length to those cited herein might be compared by insertion of gaps into the alignment, such gaps being determined, for example, by the comparison algorithm used by GAP. "*Hybridisation*" is used herein to denote the pairing of complementary nucleotide sequences to produce a DNA-DNA hybrid or a DNA-RNA hybrid. Complementary base sequences are those sequences that are related by the base-pairing rules. In DNA, A pairs with T and C pairs with G. In RNA U pairs with A and C pairs with G. In this regard, the terms "match" and "mismatch" as used herein refer to the hybridisation potential of paired nucleotides in complementary nucleic acid strands. Matched nucleotides hybridise efficiently, such as the classical A-T and G-C base pair mentioned above. Mismatches are other combinations of nucleotides that do not hybridise efficiently.

Reference herein to *"immuno-interactive"* includes reference to any interaction, reaction, or other form of association between molecules and in particular where one of the molecules is, or mimics, a component of the immune system.

By *"isolated"* is meant material that is substantially or essentially free from components that normally accompany it in its native state. For example, an "isolated polynucleotide", as used herein, refers to a polynucleotide, which has been purified from

the sequences which flank it in a naturally occurring state, *e.g.*, a DNA fragment which has been removed from the sequences which are normally adjacent to the fragment.

By *"modulating"* is meant increasing or decreasing, either directly or indirectly, the level and/or functional activity of a target molecule. For example, an agent may indirectly modulate the said level/activity by interacting with a molecule other than the target molecule. In this regard, indirect modulation of a gene encoding a target polypeptide includes within its scope modulation of the expression of a first nucleic acid molecule, wherein an expression product of the first nucleic acid molecule modulates the expression of a nucleic acid molecule encoding the target polypeptide.

By *"obtained from"* is meant that a sample such as, for example, a nucleic acid extract or polypeptide extract is isolated from, or derived from, a particular source of the host. For example, the extract may be obtained from a tissue or a biological fluid isolated directly from the host.

The term "*oligonucleotide*" as used herein refers to a polymer composed of a multiplicity of nucleotide units (deoxyribonucleotides or ribonucleotides, or related structural variants or synthetic analogues thereof) linked via phosphodiester bonds (or related structural variants or synthetic analogues thereof). Thus, while the term "oligonucleotide" typically refers to a nucleotide polymer in which the nucleotides and linkages between them are naturally occurring, it will be understood that the term also includes within its scope various analogues including, but not restricted to, peptide nucleic acids (PNAs), phosphoramidates, phosphorothioates, methyl phosphonates, 2-O-methyl ribonucleic acids, and the like. The exact size of the molecule may vary depending on the particular application. An oligonucleotide is typically rather short in length, generally from about 10 to 30 nucleotides, but the term can refer to molecules of any length, although the term "polynucleotide" or "nucleic acid" is typically used for large oligonucleotides.

By *"operably linked"* is meant that transcriptional and translational regulatory nucleic acids are positioned relative to a polypeptide-encoding polynucleotide in such a manner that the polynucleotide is transcribed and the polypeptide is translated.

The term "*patient*" refers to patients of human or other mammal and includes any individual it is desired to examine or treat using the methods of the invention. However, it will be understood that "*patient*" does not imply that symptoms are present. Suitable

mammals that fall within the scope of the invention include, but are not restricted to, primates, livestock animals (*e.g.* sheep, cows, horses, donkeys, pigs), laboratory test animals (*e.g.* rabbits, mice, rats, guinea pigs, hamsters), companion animals (*e.g.* cats, dogs) and captive wild animals (*e.g.* foxes, deer, dingoes).

By *"pharmaceutically-acceptable carrier"* is meant a solid or liquid filler, diluent or encapsulating substance that may be safely used in topical or systemic administration.

The term "*polynucleotide*" or "*nucleic acid*" as used herein designates mRNA, RNA, cRNA, cDNA or DNA. The term typically refers to oligonucleotides greater than 30 nucleotides in length. Polynucleotide sequences are understood to encompass complementary strands as well as alternative backbones described herein.

The terms *"polynucleotide variant"* and *"variant"* refer to polynucleotides displaying substantial sequence identity with a reference polynucleotide sequence or polynucleotides that hybridise with a reference sequence under stringent conditions that are defined hereinafter. These terms also encompasses polynucleotides in which one or more nucleotides have been added or deleted, or replaced with different nucleotides. In this regard, it is well understood in the art that certain alterations inclusive of mutations, additions, deletions and substitutions can be made to a reference polynucleotide whereby the altered polynucleotide retains the biological function or activity of the reference polynucleotide. The terms *"polynucleotide variant"* and *"variant"* also include naturally occurring allelic variants.

"*Polypeptide*", "*peptide*" and "*protein*" are used interchangeably herein to refer to a polymer of amino acid residues and to variants and synthetic analogues of the same. Thus, these terms apply to amino acid polymers in which one or more amino acid residues is a synthetic non-naturally occurring amino acid, such as a chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally-occurring amino acid polymers.

The term *"polypeptide variant"* refers to polypeptides which vary from a reference polypeptide by the addition, deletion or substitution of at least one amino acid. It is well understood in the art that some amino acids may be changed to others with broadly similar properties without changing the nature of the activity of the polypeptide. Accordingly, polypeptide variants as used herein encompass polypeptides that have similar

activities to a parent adhesin polypeptide. Preferred variant polypeptides comprise conservative amino acid substitutions. Exemplary conservative substitutions in a polypeptide may be made according to TABLE A:

TABLE A

Original Residue	Exemplary Substitutions
Ala	Ser
Arg	Lys
Asn	Gln, His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
Gly	Pro
His	Asn, Gln
Ile	Leu, Val
Leu	Ile, Val
Lys	Arg, Gln, Glu
Met	Leu, Ile,
Phe	Met, Leu, Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp, Phe
Val	Ile, Leu

Substantial changes in function are made by selecting substitutions that are less conservative than those shown in TABLE A. Other replacements would be non-

conservative substitutions and relatively fewer of these may be tolerated. Generally, the substitutions which are likely to produce the greatest changes in a polypeptide's properties are those in which (a) a hydrophilic residue (*e.g.*, Ser or Asn) is substituted for, or by, a hydrophobic residue (*e.g.*, Ala, Leu, Ile, Phe or Val); (b) a cysteine or proline is substituted for, or by, any other residue; (c) a residue having an electropositive side chain (*e.g.*, Arg, His or Lys) is substituted for, or by, an electronegative residue (*e.g.*, Glu or Asp) or (d) a residue having a smaller side chain (*e.g.*, Ala, Ser) or no side chain (*e.g.*, Gly) is substituted for, or by, one having a bulky side chain (*e.g.*, Phe or Trp).

By "*primer*" is meant an oligonucleotide which, when paired with a strand of DNA, is capable of initiating the synthesis of a primer extension product in the presence of a suitable polymerising agent. The primer is preferably single-stranded for maximum efficiency in amplification but may alternatively be double-stranded. A primer must be sufficiently long to prime the synthesis of extension products in the presence of the polymerisation agent. The length of the primer depends on many factors, including application, temperature to be employed, template reaction conditions, other reagents, and source of primers. For example, depending on the complexity of the target sequence, the oligonucleotide primer typically contains 15 to 35 or more nucleotides, although it may contain fewer nucleotides. Primers can be large polynucleotides, such as from about 200 nucleotides to several kilobases or more. Primers may be selected to be "substantially complementary" to the sequence on the template to which it is designed to hybridise and serve as a site for the initiation of synthesis. By "substantially complementary", it is meant that the primer is sufficiently complementary to hybridise with a target nucleotide sequence. Preferably, the primer contains no mismatches with the template to which it is designed to hybridise but this is not essential. For example, non-complementary nucleotides may be attached to the 5' end of the primer, with the remainder of the primer sequence being complementary to the template. Alternatively, non-complementary nucleotides or a stretch of non-complementary nucleotides can be interspersed into a primer, provided that the primer sequence has sufficient complementarity with the sequence of the template to hybridise therewith and thereby form a template for synthesis of the extension product of the primer.

"*Probe*" refers to a molecule that binds to a specific sequence or sub-sequence or other moiety of another molecule. Unless otherwise indicated, the term "probe" typically

refers to a polynucleotide probe that binds to another nucleic acid, often called the "target nucleic acid", through complementary base pairing. Probes may bind target nucleic acids lacking complete sequence complementarity with the probe, depending on the stringency of the hybridisation conditions. Probes can be labelled directly or indirectly.

The term *"recombinant polynucleotide"* as used herein refers to a polynucleotide formed *in vitro* by the manipulation of nucleic acid into a form not normally found in nature. For example, the recombinant polynucleotide may be in the form of an expression vector. Generally, such expression vectors include transcriptional and translational regulatory nucleic acid operably linked to the nucleotide sequence.

By *"recombinant polypeptide"* is meant a polypeptide made using recombinant techniques, *i.e.*, through the expression of a recombinant polynucleotide.

By *"reporter molecule"* as used in the present specification is meant a molecule that, by its chemical nature, provides an analytically identifiable signal that allows the detection of a complex comprising an antigen-binding molecule and its target antigen. The term "reporter molecule" also extends to use of cell agglutination or inhibition of agglutination such as red blood cells on latex beads, and the like.

Terms used to describe sequence relationships between two or more polynucleotides or polypeptides include "reference sequence", "comparison window", "sequence identity", "percentage of sequence identity" and "substantial identity". A "*reference sequence*" is at least 12 but frequently 15 to 18 and often at least 25 monomer units, inclusive of nucleotides and amino acid residues, in length. Because two polynucleotides may each comprise (1) a sequence (*i.e.*, only a portion of the complete polynucleotide sequence) that is similar between the two polynucleotides, and (2) a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity. A "*comparison window*" refers to a conceptual segment of typically 12 contiguous residues that is compared to a reference sequence. The comparison window may comprise additions or deletions (*i.e.*, gaps) of about 20% or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison

window may be conducted by computerised implementations of algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive Madison, WI, USA) or by inspection and the best alignment (*i.e.*, resulting in the highest percentage homology over the comparison window) generated by any of the various methods selected. Reference also may be made to the BLAST family of programs as for example disclosed by Altschul *et al.*, 1997. A detailed discussion of sequence analysis can be found in Unit 19.3 of Ausubel *et al.*, "Current Protocols in Molecular Biology", John Wiley & Sons Inc, 1994-1998, Chapter 15.

The term *"sperm donor"* as used herein refers in its broadest sense to any donor whose perm is used, or desired to be used, for fertilisation whether by natural or artificial means and shall include the patient's sexual partner or any other individual.

The term *"sequence identity"* as used herein refers to the extent that sequences are identical on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a *"percentage of sequence identity"* is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, I) or the identical amino acid residue (*e.g.*, Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. For the purposes of the present invention, *"sequence identity"* will be understood to mean the "match percentage" calculated by the DNASIS computer program (Version 2.5 for windows; available from Hitachi Software engineering Co., Ltd., South San Francisco, California, USA) using standard defaults as used in the reference manual accompanying the software.

"Stringency" as used herein, refers to the temperature and ionic strength conditions, and presence or absence of certain organic solvents, during hybridisation and washing procedures. The higher the stringency, the higher will be the degree of complementarity between immobilised target nucleotide sequences and the labelled probe polynucleotide sequences that remain hybridised to the target after washing.

"Stringent conditions" refers to temperature and ionic conditions under which only nucleotide sequences having a high frequency of complementary bases will hybridise. The stringency required is nucleotide sequence dependent and depends upon the various components present during hybridisation and subsequent washes, and the time allowed for these processes. Generally, in order to maximise the hybridisation rate, non-stringent hybridisation conditions are selected; about 20 to 25° C lower than the thermal melting point (T_m). The T_m is the temperature at which 50% of specific target sequence hybridises to a perfectly complementary probe in solution at a defined ionic strength and pH. Generally, in order to require at least about 85% nucleotide complementarity of hybridised sequences, highly stringent washing conditions are selected to be about 5 to 15° C lower than the T_m. In order to require at least about 70% nucleotide complementarity of hybridised sequences, moderately stringent washing conditions are selected to be about 15 to 30° C lower than the T_m. Highly permissive (low stringency) washing conditions may be as low as 50° C below the T_m, allowing a high level of mis-matching between hybridised sequences. Those skilled in the art will recognise that other physical and chemical parameters in the hybridisation and wash stages can also be altered to affect the outcome of a detectable hybridisation signal from a specific level of homology between target and probe sequences. Other examples of stringency conditions are described in section 3.3.

By *"therapeutically effective amount"*, or *"effective amount"* in the context of treating a condition described herein, is meant the administration of that amount of modulatory agent that modulates the expression of a gene encoding the ureaplasma adhesin as broadly described above to an individual in need of such treatment, either in a single dose or as part of a series, that is effective for treatment of that condition. The effective amount will vary depending upon the health and physical condition of the individual to be treated, the taxonomic group of individual to be treated, the formulation of the composition, the assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

By *"vector"* is meant a nucleic acid molecule, preferably a DNA molecule derived, for example, from a plasmid, bacteriophage, or plant virus, into which a nucleic acid sequence may be inserted or cloned. A vector preferably contains one or more unique restriction sites and may be capable of autonomous replication in a defined host cell

including a target cell or tissue or a progenitor cell or tissue thereof, or be integrable with the genome of the defined host such that the cloned sequence is reproducible. Accordingly, the vector may be an autonomously replicating vector, *i.e.*, a vector that exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, *e.g.*, a linear or closed circular plasmid, an extrachromosomal element, a minichromosome, or an artificial chromosome. The vector may contain any means for assuring self-replication. Alternatively, the vector may be one which, when introduced into the host cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated. A vector system may comprise a single vector or plasmid, two or more vectors or plasmids, which together contain the total DNA to be introduced into the genome of the host cell, or a transposon. The choice of the vector will typically depend on the compatibility of the vector with the host cell into which the vector is to be introduced. The vector may also include a selection marker such as an antibiotic resistance gene that can be used for selection of suitable transformants. Examples of such resistance genes are well known to those of skill in the art.

2. Ureaplasmas of the invention

The present invention is predicated in part on the determination that certain strains or serotypes of *Ureaplasma*, which adhere more strongly to spermatozoa than other strains or serotypes of this genus, are more likely to impact adversely on male fertility and on pregnancy outcomes, including ART pregnancy outcomes such as, but not limited to, reduced oocyte fertilisation rate, reduced viable pregnancy rate, reduced blastocyst culture rate and increased miscarriage rate. Accordingly, the invention broadly contemplates methods of ascertaining the propensity (a) for male fertility, or (b) for a lower rate of oocyte fertilisation, or (c) for an adverse pregnancy outcome or (d) for an adverse ART outcome, by detecting a strongly adherent ureaplasma according to the invention in a biological sample obtained from a patient or from a patient's sperm donor. The invention also contemplates improving male fertility or enhancing the propensity of a favourable pregnancy outcome, or for enhancing the propensity of a favourable ART outcome by treating a patient, or the sperm of a patient, with an agent that reduces the level and/or functional activity of said adhesin or that treats or prevents infection by ureaplasmas expressing said adhesin, as described hereinafter.

Accordingly, the invention provides, in one aspect, a method for detecting an ureaplasma associated with a function, activity or condition selected from a higher rate of male infertility, a lower rate of oocyte fertilisation, an adverse pregnancy outcome and an adverse assisted reproductive technology (ART) outcome. The method comprises separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm, whereby detection of ureaplasma that remains adherent after the separation indicates an association of that ureaplasma with said function, activity or condition.

Male infertility can be tested by analysis of sperm viability properties including, but not restricted to, sperm motility, sperm numbers, percentage of living sperm recovered, sperm membrane function, sperm penetration rate, sperm *in vitro* fertilisation rate and/or oocyte development following fertilisation as, for example, described *infra*.

Adverse pregnancy outcomes associated with a ureaplasma of the invention include, but are not limited to, miscarriage, pre-term delivery, premature onset of labour, prolonged rupture of membranes, neonatal morbidity and mortality.

An adverse ART outcome is suitably selected from reduced oocyte fertilisation rate, reduced viable pregnancy rate, reduced blastocyst culture rate and increased miscarriage rate.

Any suitable separation technique can be employed for separating sperm from non-sperm components, which separation permits the continuation of adherence of *Ureaplasma parvum* serotype 6 to the sperm but which does not permit the continuation of adherence of *U. parvum* serotype 1 to the sperm. For example, sperm can be separated from non-sperm substances and/or non viable sperm by allowing the motile sperm to swim away from the debris (sperm swim-up), by centrifuging the sperm through a gradient and collecting a pellet of live sperm (washing), or by passing the sperm through a column that binds the dead or unhealthy sperm. Suitable methods and compositions for washing and separating sperm are described for example by Trounson and Gardner (Handbook of *In Vitro* Fertilization, CRC Press, Boca Raton, 1994, pp. 46-50). Alternatively, reference may be made: to Bongso *et al.*, (1989, *Fertility and Sterility*, **51**: 850-854) who a method for improving concentration and motility of sperm by Ficoll separation; to Ericsson (U.S. Pat. No. 4,007,087) who discloses fractionating sperm in a medium including soluble materials such as proteins, peptides and dextran; to Shrimpton (U.S. Pat. No. 4,327,177) who discloses the separation of sperm by density in a nutrient media derived from mammalian milk; to Shrimpton (U.S. Pat. No. 4,605,558) who discloses a method of separating X and Y sperm in a density gradient and an osmolality gradient in a medium derived from milk; to Ellington *et al.* (U.S. Pat. No. 6,171,778), who describe the use of an arabinogalactan for separating motile sperm; and to Zavos *et al.* (U.S. Pat. No. 5,908,380) who describe a compartmentalised swim-up column for the swimming-up of spermatozoa, at desired levels of dilution in a media, and a swimming down of spermatozoa into compartments within the column, as well as a method for the harvesting of semen samples having particular sperm morphology, motility, progressive motility, speed, sperm concentration, fertilisation potential, and a sex ratio. Preferred separation methods include gradient centrifugation methods and sperm swim-up protocols, as for example described in Examples 3 and 5.

As used herein, the phrase *"conditions which permit the continuation of adherence"* shall mean separation conditions that permit the continuation of adherence to a sperm sample of at least 90%, preferably at least 80%, more preferably at least 70%, even more preferably at least 60%, even more preferably at least 50%, and still even more preferably at least 40% of ureaplasmas which adhered to the sperm sample before separation. By contrast, the phrase *"conditions which do not permit the continuation of adherence"* shall mean separation conditions that permit the continuation of adherence to a sperm sample of no more than 30%, preferably no more than 25%, more preferably no more than 20%, even more preferably no more than 15%, and still even more preferably no more than 10% of ureaplasmas which adhered to the sperm sample before separation.

From the foregoing, it will be understood that the invention contemplates any strain or serotype of *Ureaplasma* that remains adherent to sperm after subjecting the sperm to the conditions of separation defined herein. A ureaplasma of the invention extends to a ureaplasma that infect humans and/or animals. For example, the ureaplasma may be selected from the species *U. urealyticum, U. parvum and U. diversum*. In a preferred embodiment, the ureaplasma is a strain or serotype of *U. parvum* or *U. urealyticum*. In an especially preferred embodiment of this type, the ureaplasma is *U. parvum,* serotype 6.

The conditions of separation preferably protect one or more viability properties of the sperm such as sperm motility, sperm numbers, percentage of living sperm recovered, sperm membrane function, sperm penetration rate, sperm *in vitro* fertilisation rate and/or oocyte development following fertilisation. Any method for qualitatively or quantitatively determining sperm viability properties is contemplated by the present invention. For example, sperm numbers in a suspension can be determined by manual or computerised methods. Using computerised methods, a sperm suspension is applied to a counting chamber available in the art such as a Makler™ (Fertility Technology, Natick, Mass.) counting chamber, and the number of sperm is counted, which is equal the number of sperm/mL in the original suspension. Sperm morphology or shape is determined, for example, by smearing 10 μL of a sperm sample onto a slide and staining with a differential stain such as Wright Giemsa at 0.1% (w/v) for 30 min. Sperm then are observed under a microscope and categorised as to normal or abnormal shapes (morphology), as described in Kruger *et al.* (1987, *Urology* **30**: 248). Motility of sperm is expressed as the total percent of motile sperm, or the speed of the sperm that are motile and can be determined using

methods available in the art, such as by subjective visual determination using a phase contrast microscope, or using a computer automated semen analyser. Using phase contrast microscopy, the sample is analysed visually to group sperm into total percent motile (swimming), and total percent progressively motile (swimming forward), or the speed of the sperm which are progressively motile, *i.e.*, fast, medium, or slow. Using a computer, the track speed of individual sperm is analysed. Data is expressed as the percent motile, as well as the mean path velocity and track speed of sperm in the sample. Sperm viability as a measurement of the percentage of living sperm is determined by membrane exclusion stains available in the art. Sperm membrane function of live sperm is tested by placing sperm into a low salt (hypo-osmotic) solution. This causes sperm with healthy membranes to pump salt out of the cell, and causes the membranes of the sperm to shrink as the cell grows smaller. The sperm tail then curls inside this tighter membrane. Sperm with a curled tail are the sperm which are healthy and have functional membranes. The number of sperm with a curled tail then is expressed as a percent of the total number of sperm present. To assay sperm penetration, the ability of capacitated sperm to penetrate a dead zona free hamster egg is measured. Sperm *in vitro* fertilisation rates are determined by measuring the percent of oocytes fertilised *in vitro* using methods available in the art. The capacitated sperm sample is incubated with oocytes, and at the end of the incubation, the percentage of oocytes fertilised is determined, or the fertilised oocytes are left in culture, division occurs and the number of cleaving embryos is determined.

Detection of ureaplasma can be carried out at the functional or physical level. For example, reference may be made to standard microbiological techniques that generally identify ureaplasma by observing the hydrolysis of urea. These techniques usually involve inoculating both a complex broth medium and an agar medium containing urea and other nutrients with a freshly obtained specimen (Brunner *et al.*, 1983, *Yale J. Biol. Med.* **56**: 545; Shephard and Lunceford, 1978, *J. Clin. Micro.* **8**: 566-574). References concerning physical detection of ureaplasma include the following: Stemke and Robertson (1985, *Diagn. Microbiol. Infect. Dis.* **31**: 311) who disclose fourteen serotypes of *U. urealyticum*; Harasawa *et al.* (1990, Abstract S30-6 *UIMS Meeting*, Osaka Japan), Robertson *et al.* (1993, *J. Clin. Microbiol.* **31**: 824), Hammond *et al.* (1991, Abstract D17. Session 60, *American Society for Microbiology General Meeting*), who teach that the fourteen serotypes can be divided into at least two subspecies ("biotypes") and Kong *et al.* 2000, *Int J Syst Evol Microbiol* **50** (5): 1921-1929) who disclose that the two biotypes are separated

into 2 different species Biovar 1 (parvo biovar), which is now *U. parvum* serovars 1,3, 6, and 14 and Biovar 2, (T960 biovar), which is now *U. urealyticum* serovar 2,4, 5, 7-13, based upon restriction fragment length polymorphism (RFLP) of *U. urealyticum* genomic or based upon rRNA sequences; Roberts *et al.* (1987, *Israel J. Med. Sci.* **23**: 618) who describe the use of whole chromosomal DNA probes for detection of ureaplasma in genital specimens; Ohse and Gobel (1987, *Israel J. Med. Sci.* **23**: 352) who describe hybridisation of *U. urealyticum* rRNA genes to cloned DNA of the E. coli rRNA operon; Gobel and Stanbridge (EP-A-0 250 662) who mention biological probes for detecting Mycoplasmas or prokaryotes in general, or specific Mycoplasma and eubacterial species; Gonzales *et al.* (1991, *American Society for Microbiology Annual Meeting*, Abstract D-16) who mention a method to detect ureaplasma using a DNA probe directed to rRNA; Lee *et al.* (1992, *Arthritis and Rheumatism* **35**: 43) and Willoughby *et al.* (1991, *Infection and Immunity* **59**: 2463), who describe a procedure for detecting the *U. urealyticum* urease gene utilising PCR; Brogan *et al.* (1992, *Molecular and Cellular Probes* **6**: 411) who describe the amplification of a 186 base pair genomic *U. urealyticum* DNA fragment; Robertson *et al.* (1993, *supra*) who describe a technique involving the polymerase chain reaction (PCR) using biotype specific primers to 16S rRNA gene sequences to distinguish two *U. urealyticum* biotypes; and Hogan *et al.* (U.S. Pat. No. 6,093,538) who teach hybridisation probes which can distinguish the genus *Ureaplasma,* including clinically significant *U. urealyticum* serotypes, from their known closest phylogenetic neighbours (Mycoplasma) and from other microorganism inhabitants of the human urogenital tract and which function by hybridising to target *U. urealyticum* rRNA and/or rRNA gene sequences under stringent hybridisation assay conditions. Preferably, a ureaplasma is detected using the nested PCR technique disclosed herein, which distinguishes strain or serotypes of ureaplasma based on the nucleotide sequence of their respective multiple band antigen (*mba*) genes. Alternatively, the PCR method of Teng *et al.* (1994, *J. Clin. Micro* **32**: 1464-1469) may be employed, which uses primers based on the *mba* gene, and which distinguishes species of ureaplasma by the size of the amplified fragment. 403-404bp for *U. parvum*, 448 bp for *U. urealyticum.*

The positive detection of an ureaplasma which adheres strongly to spermatozoa is indicative that a patient has a propensity (a) for male fertility, or (b) for a lower rate of oocyte fertilisation, or (c) for an adverse pregnancy outcome or (d) for an adverse ART outcome.

3. Isolation of adhesin

From the foregoing, it is believed that the enhanced adherence to, or affinity for, spermatozoa displayed by the ureaplasmas of the invention is mediated by one or more adhesins present on their surface. Accordingly, in another aspect, the invention contemplates an isolated adhesin of a ureaplasma that remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to the sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to the sperm.

Any method suitable for detecting protein--protein interactions may be employed for identifying adhesin proteins. Among the traditional methods which may be employed are co-immunoprecipitation, crosslinking and co-purification through gradients or chromatographic columns. For example, an antigen-binding molecule (*e.g.*, a monoclonal antibody), which is immuno-interactive specifically with a ureaplasma according to the invention, can be used to isolate the adhesin from a membrane fraction of the ureaplasma.

Alternatively, one can take advantage of the property that the adhesins of many pathogens typically bind to specific carbohydrate moieties, enabling colonisation and infection. These carbohydrate moieties may be present in either glycolipids or glycoproteins. For example, many pathogens recognise gangliotriaosylceramide (GalNAcβ1-4galβ1-4glc cer [Gg_3]), gangliotetraosylceramide (galβ1-3galNAc.β1-4galβ1-4glc cer [Gg_4]), sulfatoxygalactosylceramide (SGC), sulfatoxygalactosylceramide (SGC) and phosphatidylethanolamine (PE). Thus, an affinity matrix comprising a carbohydrate (glycolipid) moiety can be prepared and used to isolate an adhesin from an organism of interest as, for example, disclosed by Lingwood (U.S. Pat. No. 6,218,147). Preferably, the ureaplasma adhesin is isolated using SGG and/or SCG in concert with a plasmon resonance technique as for example described in Example 13.

Once isolated, at least a portion of the amino acid sequence of the adhesin can be ascertained using techniques well known to those of skill in the art, such as via the Edman degradation technique (see, *e.g.*, Creighton, 1983, "Proteins: Structures and Molecular Principles", W.H. Freeman & Co., N.Y., pp.34-49) and/or mass spectrometry. The amino acid sequence obtained may be used as a guide for the generation of oligonucleotide mixtures that can be used to screen for a gene sequence encoding the adhesin. Screening may be accomplished, for example, by standard hybridisation or PCR techniques.

Techniques for the generation of oligonucleotide mixtures and the screening are well-known. (See, *e.g.*, Ausubel, *supra.*, and *PCR Protocols: A Guide to Methods and Applications*, 1990, Innis, M. *et al.*, eds. Academic Press, Inc., New York).

Additionally, methods may be employed which result in the simultaneous identification of genes which encode the adhesin. These methods include, for example, probing expression libraries with a labelled antigen-binding molecule that is immuno-interactive specifically with an ureaplasma according to the invention. Alternatively, reference may be made to Renner *et al.* (U.S. Pat. No. 6,197,502) who describe a method for identifying nucleic acids encoding proteins with a predetermined property of interest, including a particular cellular localisation, structure, enzymatic function, or affinity to other molecules. In a first step, a plurality of eukaryotic host cells is provided, wherein each host cell has an expression system comprising a different member, each member comprising a recombinant nucleic acid encoding an exogenous protein operatively linked to a control element. In a second step, the eukaryotic host cells are cultured under conditions where the exogenous protein is expressed while expression of endogenous proteins of the eukaryotic host cell is suppressed. In this time window, the exogenous protein may optionally be labelled, or may be treated in a way that allows discrimination from the untreated exogenous proteins. Finally, the member or members of the expression system that encode the exogenous protein or proteins having the property of interest (sperm binding) are identified. Accordingly, one may select for cells expressing an exogenous protein based on the exogenous protein being a membrane protein with an extracellular domain. For example, one may express exogenous proteins in cells while expression of endogenous proteins is inhibited or while using an expression system the operation of which inhibits the expression of endogenous proteins. Using this setup, one may, for example, treat the cells with proteases after inhibition of expression of endogenous proteins has set in, but while expression of exogenous protein is still continuing. After the cell surface has been deprived of extracellular proteinaceous protrusions, *i.e.*, cellular membrane receptors and so on, the expression of the exogenous protein may replenish such protrusions. However, such replenishment will only occur if the exogenous protein expressed in a particular cell is a membrane receptor molecule. Therefore, at this step, only cells containing an expression system that encodes a membrane associated molecule will have extracellular proteinaceous protrusions. Such protrusions may be used to bind the cell to any structure that binds proteins, regardless of

whether such binding occurs specifically or non-specifically with regard to the structure of the protein that is bound. Cells bound to such a protein binding structure, may then, for example be further analysed as to the sequence of the nucleic acid encoding the exogenous protein. A variety of structures that bind proteins have been described and are well known to the skilled artisan. For example, nitrocellulose, PVPF or nylon, filters may be used in this embodiment as a protein binding structure.

4. Preparation of recombinant adhesin polypeptides

Once an adhesin-encoding gene is isolated and cloned, a recombinant adhesin protein or a fragment thereof may be prepared by expressing the coding sequence of the adhesin gene or a biologically active fragment thereof in an appropriate expression system. A recombinant adhesin protein may be prepared by any suitable procedure known to those of skill in the art. For example, the recombinant adhesin polypeptide may be prepared by a procedure including the steps of:

(a) preparing a recombinant polynucleotide comprising a nucleotide sequence encoding an adhesin protein, or a biologically active fragment thereof, or a variant or derivative of these, which nucleotide sequence is operably linked to transcriptional and translational regulatory nucleic acid;

(b) introducing the recombinant polynucleotide into a suitable host cell;

(c) culturing the host cell to express recombinant polypeptide from said recombinant polynucleotide; and

(d) isolating the recombinant polypeptide.

The recombinant polynucleotide is preferably in the form of an expression vector that may be a self-replicating extra-chromosomal vector such as a plasmid, or a vector that integrates into a host genome.

The transcriptional and translational regulatory nucleic acid will generally be appropriate for the host cell used for expression. Numerous types of appropriate expression vectors and suitable regulatory sequences are known in the art for a variety of host cells. Typically, the transcriptional and translational regulatory nucleic acid may include, but is not limited to, promoter sequences, leader or signal sequences, ribosomal binding sites, transcriptional start and stop sequences, translational start and termination sequences, and

enhancer or activator sequences. Constitutive or inducible promoters as known in the art are contemplated by the invention. The promoters may be either naturally occurring promoters, or hybrid promoters that combine elements of more than one promoter.

In a preferred embodiment, the expression vector contains a selectable marker gene to allow the selection of transformed host cells. Selection genes are well known in the art and will vary with the host cell used.

The expression vector may also include a fusion partner (typically provided by the expression vector) so that the recombinant polypeptide of the invention is expressed as a fusion polypeptide with said fusion partner. The main advantage of fusion partners is that they assist identification and/or purification of said fusion polypeptide. In order to express said fusion polypeptide, it is necessary to ligate a polynucleotide according to the invention into the expression vector so that the translational reading frames of the fusion partner and the polynucleotide coincide. Well known examples of fusion partners include, but are not limited to, glutathione-S-transferase (GST), Fc potion of human IgG, maltose binding protein (MBP) and hexahistidine (HIS_6), which are particularly useful for isolation of the fusion polypeptide by affinity chromatography. For the purposes of fusion polypeptide purification by affinity chromatography, relevant matrices for affinity chromatography are glutathione-, amylose-, and nickel- or cobalt-conjugated resins respectively. Many such matrices are available in "kit" form, such as the QIAexpress™ system (Qiagen) useful with (HIS_6) fusion partners and the Pharmacia GST purification system. In a preferred embodiment, the recombinant polynucleotide is expressed in the commercial vector pFLAG as described more fully hereinafter. Another fusion partner well known in the art is green fluorescent protein (GFP). This fusion partner serves as a fluorescent "tag" which allows the fusion polypeptide of the invention to be identified by fluorescence microscopy or by flow cytometry. The GFP tag is useful when assessing subcellular localisation of the fusion polypeptide of the invention, or for isolating cells which express the fusion polypeptide of the invention. Flow cytometric methods such as fluorescence activated cell sorting (FACS) are particularly useful in this latter application. Preferably, the fusion partners also have protease cleavage sites, such as for Factor X_a or Thrombin, which allow the relevant protease to partially digest the fusion polypeptide of the invention and thereby liberate the recombinant polypeptide of the invention therefrom. The liberated polypeptide can then be isolated from the fusion partner by subsequent chromatographic separation.

Fusion partners according to the invention also include within their scope "epitope tags", which are usually short peptide sequences for which a specific antibody is available. Well known examples of epitope tags for which specific monoclonal antibodies are readily available include c-Myc, influenza virus, haemagglutinin and FLAG tags.

The step of introducing into the host cell the recombinant polynucleotide may be effected by any suitable method including transfection, and transformation, the choice of which will be dependent on the host cell employed. Such methods are well known to those of skill in the art.

Recombinant polypeptides of the invention may be produced by culturing a host cell transformed with an expression vector containing nucleic acid encoding a polypeptide, biologically active fragment, variant or derivative according to the invention. The conditions appropriate for protein expression will vary with the choice of expression vector and the host cell. This is easily ascertained by one skilled in the art through routine experimentation. Suitable host cells for expression may be prokaryotic or eukaryotic. One preferred host cell for expression of a polypeptide according to the invention is a bacterium. The bacterium used may be *Escherichia coli*. Alternatively, the host cell may be an insect cell such as, for example, *SF9* cells that may be utilised with a baculovirus expression system.

The recombinant protein may be conveniently prepared by a person skilled in the art using standard protocols as for example described in Sambrook, *et al.*, MOLECULAR CLONING. A LABORATORY MANUAL (Cold Spring Harbor Press, 1989), in particular Sections 16 and 17; Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (John Wiley & Sons, Inc. 1994-1998), in particular Chapters 10 and 16; and Coligan *et al.*, CURRENT PROTOCOLS IN PROTEIN SCIENCE (John Wiley & Sons, Inc. 1995-1997), in particular Chapters 1, 5 and 6.

Alternatively, the polypeptide, fragments, variants or derivatives of the invention may be synthesised using solution synthesis or solid phase synthesis as described, for example, in Chapter 9 of Atherton and Shephard (*supra*) and in Roberge *et al* (1995, *Science* **269**: 202).

The isolation of the adhesin sequence will enable one of ordinary skill in the art to identify and isolate nucleic acids which encode homologous adhesin proteins in other

organisms, particularly ureaplasmas. One of ordinary skill in the art may screen preparations of genomic or cDNA obtained from other organisms or from bacterial or other genomic or cDNA libraries using probes or PCR primers to identify homologous or variant sequences by standard hybridisation screening or PCR techniques. For example, suitable polynucleotide sequence variants may be prepared according to the following procedure:

- creating primers which are optionally degenerate wherein each comprises a portion of a reference polynucleotide encoding a reference adhesin protein, or a biologically active fragment thereof;

- obtaining a nucleic acid extract from an organism, which is preferably a ureaplasma; and

- using said primers to amplify, via nucleic acid amplification techniques, at least one amplification product from said nucleic acid extract, wherein said amplification product corresponds to a polynucleotide variant.

Suitable nucleic acid amplification techniques are well known to the skilled artisan, and include PCR as for example described by Ausubel *et al.* (*supra*); strand displacement amplification (SDA) as for example described in U.S. Patent No 5,422,252; rolling circle replication (RCR) as for example described by Liu *et al.*, (1996, *J. Am. Chem. Soc.* **118**:1587-1594; and International application WO 92/01813) and by Lizardi *et al.*, (International Application WO 97/19193); nucleic acid sequence-based amplification (NASBA) as for example described by Sooknanan *et al.*, (1994); and Q-β replicase amplification as for example described by Tyagi *et al.*, (1996, *Proc. Natl. Acad. Sci. USA* **93**: 5395-5400).

Alternatively, polynucleotide variants that are substantially complementary to a reference adhesin-encoding polynucleotide are identified by blotting techniques that include a step whereby nucleic acids are immobilised on a matrix (preferably a synthetic membrane such as nitrocellulose), followed by a hybridisation step, and a detection step. Southern blotting is used to identify a complementary DNA sequence; northern blotting is used to identify a complementary RNA sequence. Dot blotting and slot blotting can be used to identify complementary DNA/DNA, DNA/RNA or RNA/RNA polynucleotide sequences. Such techniques are well known by those skilled in the art, and have been described in Ausubel *et al.* (1994-1998, *supra*) at pages 2.9.1 through 2.9.20.

Polynucleotides encoding at least a portion of an adhesin protein of a strain or serotype of ureaplasma typified by the embodiments described herein are advantageous as nucleic acid probes for the identification of unique sequences found in different strains or serotypes of ureaplasma and for the identification of ureaplasma infection or colonisation, as for example described *infra* in Section 5.1. Such polynucleotides are also useful for identifying and isolating modulatory agents that can modulate the level and/or functional activity of adhesin protein according to the invention, as for example described in Sections 6 and 7.

The products encoded by the above polynucleotide are also useful for identifying and isolating modulatory agents that can modulate the level and/or functional activity of adhesin protein according to the invention, as for example described in Sections 6 and 7. Additionally, the products encoded by the above polynucleotides are useful as antigens for the production of ureaplasma-specific antigen-binding molecules as for example described *infra* in Section 5.2 and for vaccination against diseases, conditions or activities associated with ureaplasma, especially the strongly spermatozoal adherent ureaplasmas disclosed herein, as for example described in Section 8. The adhesin polypeptides, biologically active fragments, variants and derivatives encoded by the aforementioned polynucleotides, and peptides containing sequences corresponding to portions of the adhesin that are conserved between various isolates of ureaplasma and other bacteria that produce the adhesin, are useful in diagnosis of and immunisation against diseases or conditions associated with any organism that produces the adhesin protein, or an adhesin protein of similar binding specificity.

5. Methods of detecting an adhesin of the invention

The present invention is predicated in part on the discovery that sperm infected or colonised with a strongly adherent strain or serotype of ureaplasma, as disclosed herein, is more likely to impact adversely on male fertility and on pregnancy outcomes, including ART pregnancy outcomes, than uninfected/uncolonised sperm, or sperm infected or colonised with a weakly adherent strain or serotype of ureaplasma. Thus, the invention features a method for diagnosis or the prognostic assessment of (a) a high risk of male infertility, (b) a high risk of unsuccessful oocyte fertilisation (c) a high risk of an adverse pregnancy outcome or (d) a high risk of an adverse assisted reproductive technology (ART) outcome, comprising detecting a strongly adherent ureaplasma in a biological

sample obtained from the patient or from the patient's sperm donor or detecting an adhesin of a strongly adherent ureaplasma, or a genetic sequence encoding the adhesin. The patient, in one embodiment, is a male whose sperm is the subject of donation. Thus, a suitable biological sample, which can be used for detection of the ureaplasma of the invention includes, but is not limited to, blood, serum, plasma, saliva, urine, sweat, ascitic fluid, peritoneal fluid, synovial fluid, and cerebrospinal fluid, seminiferous tubule fluid (*e.g.*, in vasectomised men), semen, spermatozoa and seminiferous tubules. In an alternate embodiment, the patient is a female who is the subject of a fertilisation or a donation of an oocyte. A biological sample obtained from the female, which can be used for detection of the subject ureaplasma includes, but is not restricted to blood, serum, plasma, saliva, urine, sweat, ascitic fluid, peritoneal fluid, synovial fluid, and cerebrospinal fluid, amniotic fluid, vaginal secretions, endocervical secretions, respiratory secretions, endometrial lining, fallopian tube washings, placental tissue, ovarian tissue and fallopian tube tissue and oocyte tissue.

5.1 Genetic Diagnosis

One embodiment of the instant invention comprises a method for detecting a genetic sequence encoding a surface associated adhesin polypeptide of a strongly adherent ureaplasma according to the invention. Nucleic acid used in polynucleotide-based assays can be isolated from cells contained in the biological sample (ureaplasma cells), according to standard methodologies (Sambrook, *et al.*, "Molecular Cloning. A Laboratory Manual", Cold Spring Harbor Press, 1989; Ausubel *et al.*, "Current Protocols in Molecular Biology", John Wiley & Sons Inc, 1994-1998). The nucleic acid may be genomic DNA or fractionated or whole cell RNA. Where RNA is used, it may be desired to convert the RNA to a complementary DNA. In one embodiment, the RNA is whole cell RNA; in another, it is poly-A RNA. In one embodiment, the nucleic acid is amplified by a nucleic acid amplification technique. Suitable nucleic acid amplification techniques are well known to the skilled addressee, and include the polymerase chain reaction (PCR) as for example described in Ausubel *et al.* (*supra*) as well as modifications thereof including real-time PCR as for example described by Lee, *et al.* (1993, *Nucl. Acids Res.* **21**: 3761-3766), Ghosh, *et al.* (1994, *Nucl. Acids Res.* **22**: 3155-3159), Han, *et al.* (International application WO 96/21144) and Nadeau, *et al.* (U.S. Pat. No. 5,846,726); strand displacement amplification (SDA) as for example described in U.S. Patent No 5,422,252; rolling circle

replication (RCR) as for example described in Liu *et al.*, (1996) and International application WO 92/01813) and Lizardi *et al.*, (International Application WO 97/19193); nucleic acid sequence-based amplification (NASBA) as for example described by Sooknanan *et al.*, (1994, *Biotechniques* **17**:1077-1080); and Q-β replicase amplification as for example described by Tyagi *et al.*, (1996, *Proc. Natl. Acad. Sci. USA* **93**: 5395-5400).

Depending on the format, the specific nucleic acid of interest is identified in the sample directly using amplification or with a second, known nucleic acid following amplification. Next, the identified product is detected. In certain applications, the detection may be performed by visual means (*e.g.*, ethidium bromide staining of a gel). Alternatively, the detection may involve indirect identification of the product via chemiluminescence, radioactive scintigraphy of radiolabel or fluorescent label or even via a system using electrical or thermal impulse signals (Affymax Technology; Bellus, 1994, J *Macromol. Sci. Pure, Appl. Chem.*, **A31**(1): 1355-1376).

Following detection, one may compare the results seen in a given patient with a control reaction or a statistically significant reference group of normal patients. In this way, it is possible to correlate the presence or amount of an adhesin-encoding polynucleotide detected with the propensity (a) for male infertility, (b) for unsuccessful oocyte fertilisation (c) for an adverse pregnancy outcome or (d) for an adverse assisted reproductive technology (ART) outcome.

5.1.1 Primers and Probes

Primers may be provided in double-stranded or single-stranded form, although the single-stranded form is preferred. Probes, while perhaps capable of priming, are designed to bind to a target DNA or RNA and need not be used in an amplification process. In preferred embodiments, the probes or primers are labelled with radioactive species ^{32}P, ^{14}C, ^{35}S, ^{3}H, or other label), with a fluorophore (rhodamine, fluorescein) or a chemillumiscent label (luciferase).

5.1.2 Template Dependent Amplification Methods

A number of template dependent processes are available to amplify the marker sequences present in a given template sample. An exemplary nucleic acid amplification technique is the polymerase chain reaction (referred to as PCR) which is described in detail

in U.S. Pat. Nos. 4,683,195, 4,683,202 and 4,800,159, Ausubel *et al.* (*supra*), and in Innis *et al.*, ("PCR Protocols", Academic Press, Inc., San Diego Calif., 1990).

Briefly, in PCR, two primer sequences are prepared that are complementary to regions on opposite complementary strands of the marker sequence. An excess of deoxynucleoside triphosphates are added to a reaction mixture along with a DNA polymerase, *e.g.*, Taq polymerase. If the marker sequence is present in a sample, the primers will bind to the marker and the polymerase will cause the primers to be extended along the marker sequence by adding on nucleotides. By raising and lowering the temperature of the reaction mixture, the extended primers will dissociate from the marker to form reaction products, excess primers will bind to the marker and to the reaction products and the process is repeated.

A reverse transcriptase PCR amplification procedure may be performed in order to quantify the amount of mRNA amplified. Methods of reverse transcribing RNA into cDNA are well known and described in Sambrook *et al.*, 1989. Alternative methods for reverse transcription utilise thermostable, RNA-dependent DNA polymerases. These methods are described in WO 90/07641. Polymerase chain reaction methodologies are well known in the art.

Another method for amplification is the ligase chain reaction ("LCR"), disclosed in EPO No. 320 308. In LCR, two complementary probe pairs are prepared, and in the presence of the target sequence, each pair will bind to opposite complementary strands of the target such that they abut. In the presence of a ligase, the two probe pairs will link to form a single unit. By temperature cycling, as in PCR, bound ligated units dissociate from the target and then serve as "target sequences" for ligation of excess probe pairs. U.S. Pat. No. 4,883,750 describes a method similar to LCR for binding probe pairs to a target sequence.

Qβ Replicase, described in PCT Application No. PCT/US87/00880, may also be used as still another amplification method in the present invention. In this method, a replicative sequence of RNA that has a region complementary to that of a target is added to a sample in the presence of an RNA polymerase. The polymerase will copy the replicative sequence that can then be detected.

An isothermal amplification method, in which restriction endonucleases and ligases are used to achieve the amplification of target molecules that contain nucleotide 5'α-thio-triphosphates in one strand of a restriction site may also be useful in the amplification of nucleic acids in the present invention, Walker *et al.*, (1992, *Proc. Natl. Acad. Sci. U.S.A* **89**: 392-396).

Strand Displacement Amplification (SDA) is another method of carrying out isothermal amplification of nucleic acids which involves multiple rounds of strand displacement and synthesis, *i.e.*, nick translation. A similar method, called Repair Chain Reaction (RCR), involves annealing several probes throughout a region targeted for amplification, followed by a repair reaction in which only two of the four bases are present. The other two bases can be added as biotinylated derivatives for easy detection. A similar approach is used in SDA. Target specific sequences can also be detected using a cyclic probe reaction (CPR). In CPR, a probe having 3' and 5' sequences of non-specific DNA and a middle sequence of specific RNA is hybridised to DNA that is present in a sample. Upon hybridisation, the reaction is treated with RNase H, and the products of the probe identified as distinctive products that are released after digestion. The original template is annealed to another cycling probe and the reaction is repeated.

Still another amplification methods described in GB Application No. 2 202 328, and in PCT Application No. PCT/US89/01025, may be used in accordance with the present invention. In the former application, "modified" primers are used in a PCR-like, template- and enzyme-dependent synthesis. The primers may be modified by labelling with a capture moiety (*e.g.*, biotin) and/or a detector moiety (*e.g.*, enzyme). In the latter application, an excess of labelled probes are added to a sample. In the presence of the target sequence, the probe binds and is cleaved catalytically. After cleavage, the target sequence is released intact to be bound by excess probe. Cleavage of the labelled probe signals the presence of the target sequence.

Other nucleic acid amplification procedures include transcription-based amplification systems (TAS), including nucleic acid sequence based amplification (NASBA) and 3SR (Kwoh *et al.*, 1989, *Proc. Natl. Acad. Sci. U.S.A.*, **86**: 1173; Gingeras *et al.*, PCT Application WO 88/10315). In NASBA, the nucleic acids can be prepared for amplification by standard phenol/chloroform extraction, heat denaturation of a clinical sample, treatment with lysis buffer and minispin columns for isolation of DNA and RNA

or guanidinium chloride extraction of RNA. These amplification techniques involve annealing a primer which has target specific sequences. Following polymerisation, DNA/RNA hybrids are digested with RNase H while double stranded DNA molecules are heat denatured again. In either case the single stranded DNA is made fully double stranded by addition of second target specific primer, followed by polymerisation. The double-stranded DNA molecules are then multiply transcribed by an RNA polymerase such as T7 or SP6. In an isothermal cyclic reaction, the RNAs are reverse transcribed into single stranded DNA, which is then converted to double stranded DNA, and then transcribed once again with an RNA polymerase such as T7 or SP6. The resulting products, whether truncated or complete, indicate target specific sequences.

Davey *et al.*, EPO No. 329 822 disclose a nucleic acid amplification process involving cyclically synthesising single-stranded RNA ("ssRNA"), ssDNA, and double-stranded DNA (dsDNA), which may be used in accordance with the present invention. The ssRNA is a template for a first primer oligonucleotide, which is elongated by reverse transcriptase (RNA-dependent DNA polymerase). The RNA is then removed from the resulting DNA:RNA duplex by the action of ribonuclease H (RNase H, an RNase specific for RNA in duplex with either DNA or RNA). The resultant ssDNA is a template for a second primer, which also includes the sequences of an RNA polymerase promoter (exemplified by T7 RNA polymerase) 5' to its homology to the template. This primer is then extended by DNA polymerase (exemplified by the large "Klenow" fragment of E. coli DNA polymerase I), resulting in a double-stranded DNA ("dsDNA") molecule, having a sequence identical to that of the original RNA between the primers and having additionally, at one end, a promoter sequence. This promoter sequence can be used by the appropriate RNA polymerase to make many RNA copies of the DNA. These copies can then re-enter the cycle leading to very swift amplification. With proper choice of enzymes, this amplification can be done isothermally without addition of enzymes at each cycle. Because of the cyclical nature of this process, the starting sequence can be chosen to be in the form of either DNA or RNA.

Miller *et al.* in PCT Application WO 89/06700 disclose a nucleic acid sequence amplification scheme based on the hybridisation of a promoter/primer sequence to a target single-stranded DNA ("ssDNA") followed by transcription of many RNA copies of the sequence. This scheme is not cyclic, *i.e.*, new templates are not produced from the resultant

RNA transcripts. Other amplification methods include "RACE" and "one-sided PCR" (Frohman, M. A., In: "PCR Protocols: A Guide to Methods and Applications", Academic Press, N.Y., 1990; Ohara *et al.*, 1989, *Proc. Natl Acad. Sci. U.S.A.*, **86**: 5673-567).

Methods based on ligation of two (or more) oligonucleotides in the presence of nucleic acid having the sequence of the resulting "di-oligonucleotide", thereby amplifying the di-oligonucleotide, may also be used in the amplification step of the present invention. Wu *et al.*, (1989, *Genomics* **4**: 560).

5.1.3 Southern/Northern Blotting

Blotting techniques are well known to those of skill in the art. Southern blotting involves the use of DNA as a target, whereas Northern blotting involves the use of RNA as a target. Each provide different types of information, although cDNA blotting is analogous, in many aspects, to blotting or RNA species.

Briefly, a probe is used to target a DNA or RNA species that has been immobilised on a suitable matrix, often a filter of nitrocellulose. The different species should be spatially separated to facilitate analysis. This often is accomplished by gel electrophoresis of nucleic acid species followed by "blotting" on to the filter.

Subsequently, the blotted target is incubated with a probe (usually labelled) under conditions that promote denaturation and rehybridisation. Because the probe is designed to base pair with the target, the probe will bind a portion of the target sequence under renaturing conditions. Unbound probe is then removed, and detection is accomplished as described above.

Alternatively, an *in situ* hybridisation technique may be employed to detect a target nucleic acid sequence at the cellular or tissue level. An exemplary technique of this kind includes fluorescent *in situ* hybridisation (FISH) as, for example, described by Verma, *et al.* (1988, Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York, N.Y.)

5.1.4 Detection Methods

Products may be visualised in order to confirm amplification of the marker sequences. One typical visualisation method involves staining of a gel with ethidium

bromide and visualisation under UV light. Alternatively, if the amplification products are integrally labelled with radio- or fluorometrically-labelled nucleotides, the amplification products can then be exposed to x-ray film or visualised under the appropriate stimulating spectra, following separation.

In one embodiment, visualisation is achieved indirectly. Following separation of amplification products, a labelled nucleic acid probe is brought into contact with the amplified marker sequence. The probe preferably is conjugated to a chromophore but may be radiolabelled. In another embodiment, the probe is conjugated to a binding partner, such as an antibody or biotin, and the other member of the binding pair carries a detectable moiety or reporter molecule.

In one embodiment, detection is by a labelled probe. The techniques involved are well known to those of skill in the art and can be found in many standard texts on molecular protocols. See Sambrook *et al.*, 1989. For example, chromophore or radiolabel probes or primers identify the target during or following amplification.

One example of the foregoing is described in U.S. Pat. No. 5,279,721, which discloses an apparatus and method for the automated electrophoresis and transfer of nucleic acids. The apparatus permits electrophoresis and blotting without external manipulation of the gel and is ideally suited to carrying out methods according to the present invention.

In addition, the amplification products described above may be subjected to sequence analysis to identify specific kinds of variations using standard sequence analysis techniques. Within certain methods, exhaustive analysis of genes is carried out by sequence analysis using primer sets designed for optimal sequencing (Pignon *et al.*, 1994, *Hum. Mutat.* **3**: 126-132). The present invention provides methods by which any or all of these types of analyses may be used. Once an adhesin-encoding polynucleotide is isolated and its sequence determined, oligonucleotide primers may be designed to permit the amplification of sequences throughout that polynucleotide that may then be analysed by direct sequencing.

5.1.5 Kit Components

All the essential materials and reagents required for detecting and sequencing adhesin-encoding polynucleotides and variants thereof may be assembled together in a kit. The kits may also optionally include appropriate reagents for detection of labels, positive and negative controls, washing solutions, dilution buffers and the like. For example, a nucleic acid-based detection kit may include (i) a polynucleotide according to the invention (which may be used as a positive control), (ii) an oligonucleotide primer according to the invention. Also included may be enzymes suitable for amplifying nucleic acids including various polymerases (Reverse Transcriptase, Taq, Sequenase™ DNA ligase etc. depending on the nucleic acid amplification technique employed), deoxynucleotides and buffers to provide the necessary reaction mixture for amplification. Such kits also generally will comprise, in suitable means, distinct containers for each individual reagent and enzyme as well as for each primer or probe.

5.1.6 Chip Technologies

Also contemplated by the present invention are chip-based DNA technologies such as those described by Hacia *et al.* (1996, *Nature Genetics* **14**: 441-447) and Shoemaker *et al.* (1996, *Nature Genetics* **14**: 450-456). Briefly, these techniques involve quantitative methods for analysing large numbers of genes rapidly and accurately. By tagging genes with oligonucleotides or using fixed probe arrays, one can employ chip technology to segregate target molecules as high density arrays and screen these molecules on the basis of hybridisation. See also Pease *et al.* (1994, *Proc. Natl. Acad. Sci. U.S.A.* **91**: 5022-5026); Fodor *et al.* (1991, *Science* **251**: 767-773).

5.2 Protein-based diagnostics

5.2.1 Antigen-binding molecules

The invention also contemplates antigen-binding molecules that are specifically immuno-interactive with a ureaplasma of the invention and particularly with a ureaplasma adhesin of the invention, or with fragments thereof, or with variants or derivatives of these. For example, the antigen-binding molecules may comprise whole polyclonal antibodies. Such antibodies may be prepared, for example, by injecting a polypeptide, fragment, variant or derivative of the invention into a production species, which may include mice or

rabbits, to obtain polyclonal antisera. Methods of producing polyclonal antibodies are well known to those skilled in the art. Exemplary protocols which may be used are described for example in Coligan *et al.*, (1995-1997, *Current Protocols in Protein Science* (John Wiley & Sons, Inc.), and Ausubel *et al.*, (1994-1998, *supra*), in particular Section III of Chapter 11.

In lieu of the polyclonal antisera obtained in the production species, monoclonal antibodies may be produced using the standard method as described, for example, by Köhler and Milstein (1975, 1975, *Nature* **256**, 495-497), or by more recent modifications thereof as described, for example, in Coligan *et al.*, (1995-1997, *supra*) by immortalising spleen or other antibody producing cells derived from a production species which has been inoculated with one or more of the polypeptides, fragments, variants or derivatives of the invention.

The invention also contemplates as antigen-binding molecules Fv, Fab, Fab' and $F(ab')_2$ immunoglobulin fragments.

Alternatively, the antigen-binding molecule may comprise a synthetic stabilised Fv fragment. Exemplary fragments of this type include single chain Fv fragments (sFv, frequently termed scFv) in which a peptide linker is used to bridge the N terminus or C terminus of a V_H domain with the C terminus or N-terminus, respectively, of a V_L domain. ScFv lack all constant parts of whole antibodies and are not able to activate complement. Suitable peptide linkers for joining the V_H and V_L domains are those which allow the V_H and V_L domains to fold into a single polypeptide chain having an antigen binding site with a three dimensional structure similar to that of the antigen binding site of a whole antibody from which the Fv fragment is derived. Linkers having the desired properties may be obtained by the method disclosed in U.S. Patent No 4,946,778. However, in some cases a linker is absent. ScFvs may be prepared, for example, in accordance with methods outlined in Kreber *et al* (1997, *J. Immunol. Methods*; **201(1)**: 35-55). Alternatively, they may be prepared by methods described in U.S. Patent No 5,091,513, European Patent No 239,400 or the articles by Winter and Milstein (1991, *Nature* **349**:293) and Plückthun *et al* (1996, In *Antibody engineering: A practical approach*. 203-252).

Alternatively, the synthetic stabilised Fv fragment comprises a disulphide stabilised Fv (dsFv) in which cysteine residues are introduced into the V_H and V_L domains

such that in the fully folded Fv molecule the two residues will form a disulphide bond therebetween. Suitable methods of producing dsFv are described for example in (Glockscuther *et al. Biochem.* **29**: 1363-1367; Reiter *et al.* 1994[1], *Biol. Chem.* **269**: 18327-18331; Reiter *et al.* 1994[2], *Biochem. 33*: 5451-5459; Reiter *et al.* 1994[3], *Cancer Res.* **54**: 2714-2718; Webber *et al.* 1995, *Mol. Immunol.* **32**: 249-258).

Also contemplated as antigen-binding molecules are single variable region domains (termed dAbs) as for example disclosed in (Ward *et al.* 1989, *Nature* **341**: 544-546; Hamers-Casterman *et al.* 1993, *Nature.* **363**: 446-448; Davies & Riechmann, 1994, *FEBS Lett.* **339**: 285-290).

Alternatively, the antigen-binding molecule may comprise a "minibody". In this regard, minibodies are small versions of whole antibodies, which encode in a single chain the essential elements of a whole antibody. Suitably, the minibody is comprised of the V_H and V_L domains of a native antibody fused to the hinge region and CH3 domain of the immunoglobulin molecule as, for example, disclosed in U.S. Patent No 5,837,821.

In an alternate embodiment, the antigen binding molecule may comprise non-immunoglobulin derived, protein frameworks. For example, reference may be made to (Ku & Schultz, 1995, *Proc. Natl. Acad. Sci. USA*, **92**: 652-6556) which discloses a four-helix bundle protein cytochrome b562 having two loops randomised to create complementarity determining regions (CDRs), which have been selected for antigen binding.

The antigen-binding molecule may be multivalent (*i.e.*, having more than one antigen binding site). Such multivalent molecules may be specific for one or more antigens. Multivalent molecules of this type may be prepared by dimerisation of two antibody fragments through a cysteinyl-containing peptide as, for example disclosed by (Adams *et al.*, 1993, *Cancer Res.* **53**: 4026-4034; Cumber *et al.*, 1992, *J. Immunol.* **149**: 120-126). Alternatively, dimerisation may be facilitated by fusion of the antibody fragments to amphiphilic helices that naturally dimerise (Pack P. Plünckthun, 1992, 1992, *Biochem.* **31**: 1579-1584), or by use of domains (such as the leucine zippers jun and fos) that preferentially heterodimerise (Kostelny *et al.*, 1992, *J. Immunol.* **148**: 1547-1553). In an alternate embodiment, the multivalent molecule may comprise a multivalent single chain antibody (multi-scFv) comprising at least two scFvs linked together by a peptide linker. In this regard, non-covalently or covalently linked scFv dimers termed "diabodies"

may be used. Multi-scFvs may be bispecific or greater depending on the number of scFvs employed having different antigen binding specificities. Multi-scFvs may be prepared for example by methods disclosed in U.S. Patent No. 5,892,020.

The antigen-binding molecules of the invention may be used for affinity chromatography in isolating a natural or recombinant polypeptide or biologically active fragment of the invention. For example reference may be made to immunoaffinity chromatographic procedures described in Chapter 9.5 of Coligan *et al.*, (1995-1997, *supra*).

The antigen-binding molecules can be used to screen expression libraries for variant polypeptides of the invention as described herein. They can also be used to detect polypeptides, fragments, variants and derivatives of the invention as described hereinafter.

5.2.2 Immunodiagnostic assays

The above antigen-binding molecules have utility in detecting adhesins of strongly adherent ureaplasmas, typified by the embodiments described herein, through techniques such as ELISAs and Western blotting. Illustrative assay strategies which can be used to detect a target polypeptide of the invention include, but are not limited to, immunoassays involving the binding of an antigen-binding molecule to the target polypeptide (*e.g.*, an adhesin polypeptide) in the sample, and the detection of a complex comprising the antigen-binding molecule and the target polypeptide. Preferred immunoassays are those that can measure the level and/or functional activity of a target molecule of the invention. Typically, an antigen-binding molecule that is immuno-interactive with a target polypeptide of the invention is contacted with a biological sample suspected of containing said target polypeptide. The concentration of a complex comprising the antigen-binding molecule and the target polypeptide is measured and the measured complex concentration is then related to the concentration of target polypeptide in the sample.

Any suitable technique for determining formation of an antigen-binding molecule-target antigen complex may be used. For example, an antigen-binding molecule according to the invention, having a reporter molecule associated therewith may be utilised in immunoassays. Such immunoassays include, but are not limited to, radioimmunoassays (RIAs), enzyme-linked immunosorbent assays (ELISAs) and immunochromatographic

techniques (ICTs), Western blotting which are well known those of skill in the art. For example, reference may be made to Coligan *et al.* (1994, *supra*) which discloses a variety of immunoassays that may be used in accordance with the present invention. Immunoassays may include competitive assays as understood in the art or as for example described *infra*. It will be understood that the present invention encompasses qualitative and quantitative immunoassays.

Suitable immunoassay techniques are described for example in US Patent Nos. 4,016,043, 4, 424,279 and 4,018,653. These include both single-site and two-site assays of the non-competitive types, as well as the traditional competitive binding assays. These assays also include direct binding of a labelled antigen-binding molecule to a target antigen.

Two site assays are particularly favoured for use in the present invention. A number of variations of these assays exist, all of which are intended to be encompassed by the present invention. Briefly, in a typical forward assay, an unlabelled antigen-binding molecule such as an unlabelled antibody is immobilised on a solid substrate and the sample to be tested brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen complex, another antigen-binding molecule, suitably a second antibody specific to the antigen, labelled with a reporter molecule capable of producing a detectable signal is then added and incubated, allowing time sufficient for the formation of another complex of antibody-antigen-labelled antibody. Any unreacted material is washed away and the presence of the antigen is determined by observation of a signal produced by the reporter molecule. The results may be either qualitative, by simple observation of the visible signal, or may be quantitated by comparing with a control sample containing known amounts of antigen. Variations on the forward assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound antibody. These techniques are well known to those skilled in the art, including minor variations as will be readily apparent. In accordance with the present invention, the sample is one that might contain an antigen including a tissue or fluid as described above.

In the typical forward assay, a first antibody having specificity for the antigen or antigenic parts thereof is either covalently or passively bound to a solid surface. The solid surface is typically glass or a polymer, the most commonly used polymers being cellulose,

polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs of microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well known in the art and generally consist of cross-linking covalently binding or physically adsorbing, the polymer-antibody complex is washed in preparation for the test sample. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period of time sufficient and under suitable conditions to allow binding of any antigen present to the antibody. Following the incubation period, the antigen-antibody complex is washed and dried and incubated with a second antibody specific for a portion of the antigen. The second antibody has generally a reporter molecule associated therewith that is used to indicate the binding of the second antibody to the antigen. The amount of labelled antibody that binds, as determined by the associated reporter molecule, is proportional to the amount of antigen bound to the immobilised first antibody.

An alternative method involves immobilising the antigen in the biological sample and then exposing the immobilised antigen to specific antibody that may or may not be labelled with a reporter molecule. Depending on the amount of target and the strength of the reporter molecule signal, a bound antigen may be detectable by direct labelling with the antibody. Alternatively, a second labelled antibody, specific to the first antibody is exposed to the target-first antibody complex to form a target-first antibody-second antibody tertiary complex. The complex is detected by the signal emitted by the reporter molecule.

From the foregoing, it will be appreciated that the reporter molecule associated with the antigen-binding molecule may include the following:

(a) direct attachment of the reporter molecule to the antigen-binding molecule;

(b) indirect attachment of the reporter molecule to the antigen-binding molecule; *i.e.*, attachment of the reporter molecule to another assay reagent which subsequently binds to the antigen-binding molecule; and

(c) attachment to a subsequent reaction product of the antigen-binding molecule.

The reporter molecule may be selected from a group including a chromogen, a catalyst, an enzyme, a fluorochrome, a chemiluminescent molecule, a lanthanide ion such as Europium (Eu^{34}), a radioisotope and a direct visual label.

In the case of a direct visual label, use may be made of a colloidal metallic or non-metallic particle, a dye particle, an enzyme or a substrate, an organic polymer, a latex particle, a liposome, or other vesicle containing a signal producing substance and the like.

A large number of enzymes suitable for use as reporter molecules is disclosed in United States Patent Specifications U.S. 4,366,241, U.S. 4,843,000, and U.S. 4,849,338. Suitable enzymes useful in the present invention include alkaline phosphatase, horseradish peroxidase, luciferase, β-galactosidase, glucose oxidase, lysozyme, malate dehydrogenase and the like. The enzymes may be used alone or in combination with a second enzyme that is in solution.

Suitable fluorochromes include, but are not limited to, fluorescein isothiocyanate (FITC), tetramethylrhodamine isothiocyanate (TRITC), R-Phycoerythrin (RPE), and Texas Red. Other exemplary fluorochromes include those discussed by Dower *et al.* (International Publication WO 93/06121). Reference also may be made to the fluorochromes described in U.S. Patents 5,573,909 (Singer *et al*), 5,326,692 (Brinkley *et al*). Alternatively, reference may be made to the fluorochromes described in U.S. Patent Nos. 5,227,487, 5,274,113, 5,405,975, 5,433,896, 5,442,045, 5,451,663, 5,453,517, 5,459,276, 5,516,864, 5,648,270 and 5,723,218.

In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodates. As will be readily recognised, however, a wide variety of different conjugation techniques exist which are readily available to the skilled artisan. The substrates to be used with the specific enzymes are generally chosen for the production of, upon hydrolysis by the corresponding enzyme, a detectable colour change. Examples of suitable enzymes include those described *supra*. It is also possible to employ fluorogenic substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labelled antibody is added to the first antibody-antigen complex. It is then allowed to bind, and excess reagent is washed away. A solution containing the appropriate substrate is then added to the complex of antibody-antigen-antibody. The substrate will react with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an indication of the amount of antigen which was present in the sample.

Alternately, fluorescent compounds, such as fluorescein, rhodamine and the lanthanide, europium (EU), may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state to excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. The fluorescent-labelled antibody is allowed to bind to the first antibody-antigen complex. After washing off the unbound reagent, the remaining tertiary complex is then exposed to light of an appropriate wavelength. The fluorescence observed indicates the presence of the antigen of interest. Immunofluorometric assays (IFMA) are well established in the art. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules may also be employed.

It will be well understood that other means of testing target polypeptide (*e.g.*, an adhesin of the invention) levels are available, including, for instance, those involving testing for an altered level of adhesin binding activity to a spermatozoon or to a carbohydrate (*e.g.*, glycolipid), or Western blot analysis of adhesin protein levels on ureaplasma or on spermatozoa, using an anti-adhesin antigen-binding molecule, or assaying the amount of antigen-binding molecule or other adhesin-binding partner which is not bound to a sample, and subtracting from the total amount of antigen-binding molecule or binding partner added.

6. Identification of target molecule modulators

The invention also features a method of screening for an agent that modulates the level and/or functional activity of a target molecule comprising an expression product of a gene selected from the group consisting of an adhesin-encoding gene and another gene relating to the same regulatory or biosynthetic pathway as the adhesin-encoding gene. The method comprises contacting a preparation comprising a first member selected from the group consisting of said expression product, and a biologically active fragment of said expression product, or a second member selected from the group consisting of a genetic sequence, which regulates or encodes said expression product, and a fragment of said genetic sequence, with a test agent, and detecting a change in the level and/or functional activity of said first member, or of an expression product relating to said second member.

Any suitable assay for detecting, measuring or otherwise determining modulation of adherence of said adhesin for a binding partner (*e.g.*, a carbohydrate or sperm) is contemplated by the present invention. Assays of a suitable nature are known to persons of skill in the art. It will be understood, in this regard, that the present invention is not limited to the use or practice of any one particular assay for determining affinity of protein--protein interactions or strength of adherence between binding partners.

Modulatory compounds contemplated by the present invention includes agonists and antagonists of adhesin gene expression. Antagonists of adhesin gene expression include antisense molecules, ribozymes and co-suppression molecules. Agonists include molecules which increase promoter activity or interfere with negative mechanisms. Agonists of an adhesin according to the invention include molecules which overcome any negative regulatory mechanism. Antagonists of adhesin polypeptides include antibodies and inhibitor peptide fragments.

Candidate agents encompass numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 50 and less than about 2,500 Dalton. Candidate agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules including, but not limited to: peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogues or combinations thereof.

Small (non-peptide) molecule modulators of an adhesin of the invention are particularly preferred. In this regard, small molecules are particularly preferred because such molecules are more readily absorbed after oral administration, have fewer potential antigenic determinants, and/or are more likely to cross the cell membrane than larger, protein-based pharmaceuticals. Small organic molecules may also have the ability to gain entry into an appropriate cell and affect the expression of a gene (*e.g.*, by interacting with the regulatory region or transcription factors involved in gene expression); or affect the activity of a gene by inhibiting or enhancing the binding of accessory molecules.

Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means, and may be used to produce combinatorial libraries. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification, etc. to produce structural analogues. Screening may also be directed to known pharmacologically active compounds and chemical analogues thereof.

Screening for modulatory agents according to the invention can be achieved by any suitable method. For example, the method may include contacting a cell comprising a polynucleotide corresponding to an adhesin gene or to a gene belonging to the same regulatory or biosynthetic pathway as the adhesin gene, with an agent suspected of having said modulatory activity and screening for the modulation of the level and/or functional activity of a protein encoded by said polynucleotide, or the modulation of the level of an expression product encoded by the polynucleotide, or the modulation of the activity or expression of a downstream cellular target of said protein or said expression product. Detecting such modulation can be achieved utilising techniques including, but not restricted to, ELISA, cell-based ELISA, filter-binding ELISA, inhibition ELISA, Western blots, immunoprecipitation, slot or dot blot assays, immunostaining, RIA, scintillation proximity assays, fluorescent immunoassays using antigen-binding molecule conjugates or antigen conjugates of fluorescent substances such as fluorescein or rhodamine, Ouchterlony double diffusion analysis, immunoassays employing an avidin-biotin or a streptavidin-biotin detection system, and nucleic acid detection assays including reverse transcriptase polymerase chain reaction (RT-PCR).

It will be understood that a polynucleotide from which a target molecule of interest is regulated or expressed may be naturally occurring in the cell which is the subject of testing or it may have been introduced into the host cell for the purpose of testing. Further, the naturally-occurring or introduced sequence may be constitutively expressed – thereby providing a model useful in screening for agents which down-regulate expression of an encoded product of the sequence wherein said down regulation can be at the nucleic acid or expression product level – or may require activation – thereby providing a model useful in screening for agents that up-regulate expression of an encoded product of the

sequence. Further, to the extent that a polynucleotide is introduced into a cell, that polynucleotide may comprise the entire coding sequence which codes for a target protein or it may comprise a portion of that coding sequence (*e.g.* a domain such as a protein binding domain) or a portion that regulates expression of a product encoded by the polynucleotide (*e.g.*, a promoter). For example, the promoter that is naturally associated with the polynucleotide may be introduced into the cell that is the subject of testing. In this regard, where only the promoter is utilised, detecting modulation of the promoter activity can be achieved, for example, by operably linking the promoter to a suitable reporter polynucleotide including, but not restricted to, green fluorescent protein (GFP), luciferase, β-galactosidase and catecholamine acetyl transferase (CAT). Modulation of expression may be determined by measuring the activity associated with the reporter polynucleotide.

In another example, the subject of detection could be a downstream regulatory target of the target molecule, rather than target molecule itself or the reporter molecule operably linked to a promoter of a gene encoding a product the expression of which is regulated by the target protein.

These methods provide a mechanism for performing high throughput screening of putative modulatory agents such as proteinaceous or non-proteinaceous agents comprising synthetic, combinatorial, chemical and natural libraries. These methods will also facilitate the detection of agents which bind either the polynucleotide encoding the target molecule or which modulate the expression of an upstream molecule, which subsequently modulates the expression of the polynucleotide encoding the target molecule. Accordingly, these methods provide a mechanism of detecting agents that either directly or indirectly modulate the expression and/or activity of a target molecule according to the invention.

In a series of preferred embodiments, the present invention provides assays for identifying small molecules or other compounds (*i.e.*, modulatory agents) which are capable of inducing or inhibiting the level and/or or functional activity of target molecules according to the invention. The assays may be performed *in vitro* using ureaplasmas non-transformed cells (*e.g.*, ureaplasma cells), immortalised cells, or recombinant cells. In addition, the assays may detect the presence of increased or decreased expression of genes or production of proteins on the basis of increased or decreased mRNA expression (using, for example, the nucleic acid probes disclosed herein), increased or decreased levels of protein products (using, for example, the antigen binding molecules disclosed herein), or

increased or decreased levels of expression of a reporter gene (*e.g.*, GFP, β-galactosidase or luciferase) operatively linked to a target molecule-related gene regulatory region in a recombinant construct.

Thus, for example, one may culture cells which produce a particular target molecule and add to the culture medium one or more test compounds. After allowing a sufficient period of time (*e.g.*, 6-72 hours) for the compound to induce or inhibit the level and/or functional activity of the target molecule, any change in said level from an established baseline may be detected using any of the techniques described above and well known in the art. Using the nucleic acid probes and/or antigen-binding molecules prepared for example according to protocols described herein, detection of changes in the level and or functional activity of a target molecule, and thus identification of the compound as agonist or antagonist of the target molecule, requires only routine experimentation.

In particularly preferred embodiments, a recombinant assay is employed in which a reporter gene encoding, for example, GFP, β-galactosidase or luciferase is operably linked to the 5' regulatory regions of a target molecule related gene. Such regulatory regions may be easily isolated and cloned by one of ordinary skill in the art in light of the present disclosure. The reporter gene and regulatory regions are joined in-frame (or in each of the three possible reading frames) so that transcription and translation of the reporter gene may proceed under the control of the regulatory elements of the target molecule related gene. The recombinant construct may then be introduced into any appropriate cell type. The transformed cells may be grown in culture and, after establishing the baseline level of expression of the reporter gene, test compounds may be added to the medium. The ease of detection of the expression of the reporter gene provides for a rapid, high throughput assay for the identification of agonists or antagonists of the target molecules of the invention.

Compounds identified by this method will have potential utility in modifying the expression of target molecule related genes *in vivo*. These compounds may be further tested in the animal models to identify those compounds having the most potent *in vivo* effects. In addition, as described above with respect to small molecules having target polypeptide binding activity, these molecules may serve as "lead compounds" for the further development of pharmaceuticals by, for example, subjecting the compounds to

sequential modifications, molecular modelling, and other routine procedures employed in rational drug design.

In another embodiment, a method of identifying agents that inhibit the adhesion of an adhesin of the invention is provided in which a purified preparation of adhesin protein is incubated in the presence and absence of a candidate agent, and the affinity of the adhesin for a spermatozoon or for a carbohydrate binding partner is measured by a suitable assay. For example, an adhesin inhibitor can be identified by measuring the ability of a candidate agent to decrease the level of the adhesin on the surface of a cell (*e.g.*, a ureaplasma cell). In this method, a cell that is capable of expressing an adhesin-encoding gene is exposed to, or cultured in the presence and absence of, the candidate agent and the binding of the cell to a spermatozoon or to a carbohydrate binding partner is detected. An agent tests positive if it inhibits this binding.

In yet another embodiment, random peptide libraries consisting of all possible combinations of amino acids attached to a solid phase support may be used to identify peptides that are able to bind to a target molecule or to a functional domain thereof. Identification of molecules that are able to bind to a target molecule may be accomplished by screening a peptide library with a recombinant soluble target molecule. The target molecule may be purified, recombinantly expressed or synthesised by any suitable technique. Such molecules may be conveniently prepared by a person skilled in the art using standard protocols as for example described in Sambrook, *et al.*, MOLECULAR CLONING. A LABORATORY MANUAL (Cold Spring Harbor Press, 1989), in particular Sections 16 and 17; Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (John Wiley & Sons, Inc. 1994-1998), in particular Chapters 10 and 16; and Coligan *et al.*, CURRENT PROTOCOLS IN PROTEIN SCIENCE (John Wiley & Sons, Inc. 1995-1997), in particular Chapters 1, 5 and 6. Alternatively, a target polypeptide according to the invention may be synthesised using solution synthesis or solid phase synthesis as described, for example, in Chapter 9 of Atherton and Shephard (*supra*) and in Roberge *et al* (1995, *Science* **269**: 202).

To identify and isolate the peptide/solid phase support that interacts and forms a complex with a target molecule, preferably a target polypeptide, it may be necessary to label or "tag" the target polypeptide. The target polypeptide may be conjugated to any suitable reporter molecule, including enzymes such as alkaline phosphatase and

horseradish peroxidase and fluorescent reporter molecules such as fluorescein isothyiocynate (FITC), phycoerythrin (PE) and rhodamine. Conjugation of any given reporter molecule, with target polypeptide, may be performed using techniques that are routine in the art. Alternatively, target polypeptide expression vectors may be engineered to express a chimeric target polypeptide containing an epitope for which a commercially available antigen-binding molecule exists. The epitope specific antigen-binding molecule may be tagged using methods well known in the art including labelling with enzymes, fluorescent dyes or coloured or magnetic beads.

For example, the "tagged" target polypeptide conjugate is incubated with the random peptide library for 30 minutes to one hour at 22° C to allow complex formation between target polypeptide and peptide species within the library. The library is then washed to remove any unbound target polypeptide. If the target polypeptide has been conjugated to alkaline phosphatase or horseradish peroxidase the whole library is poured into a petri dish containing a substrate for either alkaline phosphatase or peroxidase, for example, 5-bromo-4-chloro-3-indoyl phosphate (BCIP) or 3,3',4,4"-diamnobenzidine (DAB), respectively. After incubating for several minutes, the peptide/solid phase-target polypeptide complex changes colour, and can be easily identified and isolated physically under a dissecting microscope with a micromanipulator. If a fluorescently tagged target polypeptide has been used, complexes may be isolated by fluorescent activated sorting. If a chimeric target polypeptide having a heterologous epitope has been used, detection of the peptide/target polypeptide complex may be accomplished by using a labelled epitope specific antigen-binding molecule. Once isolated, the identity of the peptide attached to the solid phase support may be determined by peptide sequencing.

7. Method of modulating the level and/or functional activity of an adhesin

The invention, therefore, provides a method for modulating adhesion of a ureaplasma to sperm, comprising contacting said ureaplasma with an agent for a time and under conditions sufficient to modulate the level and/or functional activity of an adhesin polypeptide as broadly described above. In a preferred embodiment, the agent decreases the level and/or functional activity of the adhesin protein. In such a case, the agent is suitably used to reduce, repress or otherwise inhibit adherence of a ureaplasma to sperm. Suitable adhesin inhibitors may be identified or produced by methods for example disclosed in Section 6.

For example, a suitable adhesin inhibitor may comprise oligoribonucleotide sequences, that include anti-sense RNA and DNA molecules and ribozymes that function to inhibit the translation of adhesin protein-encoding mRNA. Anti-sense RNA and DNA molecules act to directly block the translation of mRNA by binding to targeted mRNA and preventing protein translation. In regard to antisense DNA, oligodeoxyribonucleotides derived from the translation initiation site, *e.g.*, between -10 and +10 regions of a gene encoding a polypeptide according to the invention, are preferred. Ribozymes are enzymatic RNA molecules capable of catalysing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridisation of the ribozyme molecule to complementary target RNA, followed by a endonucleolytic cleavage. Within the scope of the invention are engineered hammerhead motif ribozyme molecules that specifically and efficiently catalyse endonucleolytic cleavage of adhesin-encoding RNA sequences.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, which include the following sequences, GUA, GUU and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for predicted structural features such as secondary structure that may render the oligonucleotide sequence unsuitable. The suitability of candidate targets may also be evaluated by testing their accessibility to hybridisation with complementary oligonucleotides, using ribonuclease protection assays.

Both anti-sense RNA and DNA molecules and ribozymes may be prepared by any method known in the art for the synthesis of RNA molecules. These include techniques for chemically synthesising oligodeoxyribonucleotides well known in the art such as for example solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors which incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesise antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

Various modifications to the DNA molecules may be introduced as a means of increasing intracellular stability and half-life. Possible modifications include but are not

limited to the addition of flanking sequences of ribo- or deoxy- nucleotides to the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the oligodeoxyribonucleotide backbone.

Alternatively, an adhesin inhibitor may comprise an antigen-binding molecule that is immuno-interactive with an extracellular portion of the adhesin of the invention. Such antigen-binding molecules can be produced by any suitable method known to persons of skill in the art. Examples of antigen-binding molecules, which are contemplated by the present invention, include those described in Section 5.2.1.

8. Compositions and therapeutic/prophylactic interventions

The adhesin polypeptides, fragments, variants and derivatives, and the polynucleotides and polynucleotide variants described in Section 3, and the modulatory agents described in Section 6 and 7 (therapeutic agents) can be used as actives for the treatment or prophylaxis of male fertility or for enhancing the propensity of a favourable pregnancy outcome, or for enhancing the propensity of a favourable ART outcome as, for example, described *infra*. These therapeutic agents can be administered to a patient either by themselves, or in pharmaceutical compositions where they are mixed with a suitable pharmaceutically acceptable carrier. Alternatively, the therapeutic agents may be used to treat a sperm sample of a patient. The patient includes, but is not restricted to, a sperm donor, an oocyte donor, a sperm recipient, an oocyte recipient and an embryo recipient.

Accordingly, the invention also provides a composition for enhancing or otherwise improving male fertility, or for enhancing the propensity for a favourable pregnancy outcome, or for enhancing the propensity for a favourable assisted reproductive technology (ART) outcome, wherein said composition comprises an agent that reduces the level and/or functional activity of an ureaplasma adhesin, wherein said ureaplasma remains adherent to sperm after separating sperm from non-sperm substances under conditions which permit the continuation of adherence of *Ureaplasma parvum* serotype 6 to said sperm but which do not permit the continuation of adherence of *U. parvum* serotype 1 to said sperm, and wherein said composition further optionally comprises a pharmaceutically acceptable carrier.

The invention further provides a method for improving male fertility, enhancing the propensity for a favourable pregnancy outcome, or for enhancing the propensity for a

favourable assisted reproductive technology (ART) outcome in a patient. The method comprises administering to a patient, or to the patient's sperm donor, as the case may be, or to the sperm of a patient or to the sperm of the patient's sperm donor, as the case may be, a therapeutically effective amount of an agent that reduces the level and/or functional activity of an ureaplasma adhesin according to the invention, and optionally together with a pharmaceutically acceptable carrier.

Therapeutic agents may be formulated and administered systemically or locally. Techniques for formulation and administration may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, Pa., latest edition. Suitable routes may, for example, include oral, rectal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections. For injection, the therapeutic agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art. Intra-muscular and subcutaneous injection is appropriate, for example, for administration of immunogenic compositions, vaccines and DNA vaccines.

The agents can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. Such carriers enable the compounds of the invention to be formulated in dosage forms such as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. These carriers may be selected from sugars, starches, cellulose and its derivatives, malt, gelatine, talc, calcium sulphate, vegetable oils, synthetic oils, polyols, alginic acid, phosphate buffered solutions, emulsifiers, isotonic saline, and pyrogen-free water.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. The dose of agent administered to a patient should be sufficient to effect a beneficial response in the patient over time such as improving male fertility or enhancing the propensity of a favourable pregnancy outcome, or for enhancing

the propensity of a favourable ART outcome. The quantity of the agent(s) to be administered may depend on the subject to be treated inclusive of the age, sex, weight and general health condition thereof. In this regard, precise amounts of the agent(s) for administration will depend on the judgement of the practitioner. In determining the effective amount of the agent to be administered in the treatment or prophylaxis of the condition, the physician may evaluate tissue levels of a polypeptide, fragment, variant or derivative of the invention, and progression or amelioration of the condition or disorder. In any event, those of skill in the art may readily determine suitable dosages of the therapeutic agents of the invention.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilisers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as., for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Such compositions may be prepared by any of the methods of pharmacy but all methods include the step of bringing into association one or more therapeutic agents as described above with the carrier which constitutes one or more necessary ingredients. In general, the pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known, *e.g.*, by means of conventional mixing, dissolving,

granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilising processes.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterise different combinations of active compound doses.

Pharmaceutical which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticiser, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilisers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilisers may be added.

Dosage forms of the therapeutic agents of the invention may also include injecting or implanting controlled releasing devices designed specifically for this purpose or other forms of implants modified to act additionally in this fashion. Controlled release of an agent of the invention may be effected by coating the same, for example, with hydrophobic polymers including acrylic resins, waxes, higher aliphatic alcohols, polylactic and polyglycolic acids and certain cellulose derivatives such as hydroxypropylmethyl cellulose. In addition, controlled release may be effected by using other polymer matrices, liposomes and/or microspheres.

Therapeutic agents of the invention may be provided as salts with pharmaceutically compatible counterions. Pharmaceutically compatible salts may be formed with many acids, including but not limited to hydrochloric, sulphuric, acetic, lactic, tartaric, malic, succinic, *etc.* Salts tend to be more soluble in aqueous or other protonic solvents that are the corresponding free base forms.

For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes

the IC50 as determined in cell culture (*e.g.*, the concentration of a test agent, which achieves a half-maximal inhibition or enhancement of ureaplasma adherence to sperm). Such information can be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of such therapeutic agents can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds that exhibit large therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilised. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See for example Fingl *et al.*, 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p1).

Dosage amount and interval may be adjusted individually to provide plasma levels of the active agent which are sufficient to maintain ureaplasma adhesin-inhibitory or enhancement effects. Usual patient dosages for systemic administration range from 1-2000 mg/day, commonly from 1-250 mg/day, and typically from 10-150 mg/day. Stated in terms of patient body weight, usual dosages range from 0.02-25 mg/kg/day, commonly from 0.02-3 mg/kg/day, typically from 0.2-1.5 mg/kg/day.

Alternately, one may administer the agent in a local rather than systemic manner, for example, *via* injection of the compound directly into a tissue, which is preferably a testicular tissue, often in a depot or sustained release formulation. Furthermore, one may administer the agent in a targeted drug delivery system, for example, in a liposome coated with tissue-specific antibody. The liposomes will be targeted to and taken up selectively by the tissue.

In another embodiment, the therapeutic agents of the invention can be used for treatment *in vitro* of spermatozoa or oocytes that are infected with ureaplasma. For example, during their preparation for an artificial reproductive technique, the spermatozoa

and/or oocytes can be treated *in vitro* with a therapeutic agent of the invention. If desired, the spermatozoa and/or oocytes may also be treated with other agents including antibiotics, which are effective against ureaplasma.

From the foregoing, it will be appreciated that the agents of the invention may be used as therapeutic or prophylactic immunomodulating compositions or vaccines. Accordingly, the invention extends to the production of immunomodulating compositions for eliciting an immunogenic response, and particularly the production of elements that specifically bind to a strongly adherent ureaplasma of the invention. The composition comprises a proteinaceous molecule selected from the group consisting of an isolated adhesin of said ureaplasma, a biologically active fragment of said adhesin, a variant of said adhesin, a variant of said biologically active fragment, a derivative of said adhesin, a derivative of said biologically active fragment, and a derivative of said variant, and/or or a vector comprising a polynucleotide encoding said proteinaceous molecule, wherein said polynucleotide is operably linked to a regulatory polynucleotide, wherein said composition optionally further comprises one or more pharmaceutically acceptable carriers, adjuvants and/or diluents.

The invention also extends to a method for treatment and/or prophylaxis of male infertility, an adverse pregnancy outcome or an adverse assisted reproductive technology (ART) outcome in a patient, comprising administering to the sperm donor or to the patient's sperm donor, as the case may be, an immunogenically effective amount of the immunopotentiating composition as broadly described above.

Any suitable procedure is contemplated for producing the immunomodulating compositions of the invention. Exemplary procedures include, for example, those described in NEW GENERATION VACCINES (1997, Levine *et al.*, Marcel Dekker, Inc. New York, Basel Hong Kong).

It will be appreciated from the foregoing that the present invention contemplates the use of nucleic acid compositions for the purpose of vaccination or immunomodulation. In this regard, a synthetic construct can be used to immunise a patient, which construct includes a polynucleotide encoding an adhesin according to the invention, wherein said polynucleotide is operably connected to one or more regulatory sequences that direct expression of said polynucleotide in said patient.

Typically, such constructs or vectors are derived from viral DNA sequences such as adenovirus, adeno-associated viruses, herpes-simplex viruses and retroviruses. Suitable immunomodulating vectors currently available to the skilled person may be found, for example, in Wu and Ataai (2000, *Curr. Opin. Biotechnol.* **11** (2): 205-208), Vigna and Naldini (2000, *J. Gene Med.* **2** (5): 308-316), Kay, *et al.* (2001, *Nat. Med.* **7** (1): 33-40), Athanasopoulos, *et al.* (2000, *Int. J. Mol. Med.* **6** (4): 363-375) and Walther and Stein (2000, *Drugs* **60** (2): 249-271).

Administration of the immunomodulating construct to a patient, preferably a human patient, may include delivery *via* direct oral intake, systemic injection, or delivery to selected tissue(s) or cells, or indirectly via delivery to cells isolated from the patient or a compatible donor. In a preferred embodiment, the immunomodulating construct is delivered intradermally. Delivery of said immunomodulating construct to cells or tissues of the patient or said compatible donor may be facilitated by microprojectile bombardment, liposome mediated transfection (*e.g.*, lipofectin or lipofectamine), electroporation, calcium phosphate or DEAE-dextran-mediated transfection, for example. A discussion of suitable delivery methods may be found in Chapter 9 of CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (Eds. Ausubel *et al.*; John Wiley & Sons Inc., 1997 Edition), for example, which is herein incorporated by reference.

The step of introducing the immunomodulating construct into a target cell or tissue will differ depending on the intended use and species, and can involve one or more of non-viral and viral vectors, cationic liposomes, retroviruses, and adenoviruses such as, for example, described in Mulligan, R.C., (1993). Such methods can include, for example:

A. Local application of the expression vector by injection (Wolff *et al.*, 1990), surgical implantation, instillation or any other means. This method can also be used in combination with local application by injection, surgical implantation, instillation or any other means, of cells responsive to the protein encoded by the expression vector so as to increase the effectiveness of that treatment. This method can also be used in combination with local application by injection, surgical implantation, instillation or any other means, of another factor or factors required for the activity of said protein.

B. General systemic delivery by injection of DNA, (Calabretta *et al.*, 1993), or RNA, alone or in combination with liposomes (Zhu *et al.*, 1993), viral capsids or

nanoparticles (Bertling *et al.*, 1991) or any other mediator of delivery. Improved targeting might be achieved by linking the polynucleotide/expression vector to a targeting molecule (the so-called "magic bullet" approach employing, for example, an antigen-binding molecule), or by local application by injection, surgical implantation or any other means, of another factor or factors required for the activity of the protein encoded by said expression vector, or of cells responsive to said protein.

C. Injection or implantation or delivery by any means, of cells that have been modified *ex vivo* by transfection (for example, in the presence of calcium phosphate: Chen *et al.*, 1987, or of cationic lipids and polyamines: Rose *et al.*, 1991), infection, injection, electroporation (Shigekawa *et al.*, 1988) or any other way so as to increase the expression of said polynucleotide in those cells. The modification can be mediated by plasmid, bacteriophage, cosmid, viral (such as adenoviral or retroviral; Mulligan, 1993; Miller, 1992; Salmons *et al.*, 1993) or other vectors, or other agents of modification such as liposomes (Zhu *et al.*, 1993), viral capsids or nanoparticles (Bertling *et al.*, 1991), or any other mediator of modification. The use of cells as a delivery vehicle for genes or gene products has been described by Barr *et al.*, 1991 and by Dhawan *et al.*, 1991. Treated cells can be delivered in combination with any nutrient, growth factor, matrix or other agent that will promote their survival in the treated subject.

Immunomodulating compositions according to the present invention can also contain a physiologically acceptable diluent or excipient such as water, phosphate buffered saline and saline. They may also include an adjuvant as is well known in the art. Suitable adjuvants include, but are not limited to: surface active substances such as hexadecylamine, octadecylamine, octadecyl amino acid esters, lysolecithin, dimethyldioctadecylammonium bromide, N, N-dicoctadecyl-N', N'bis(2-hydroxyethyl-propanediamine), methoxyhexadecylglycerol, and pluronic polyols; polyamines such as pyran, dextransulfate, poly IC carbopol; peptides such as muramyl dipeptide and derivatives, dimethylglycine, tuftsin; oil emulsions; and mineral gels such as aluminum phosphate, aluminum hydroxide or alum; lymphokines, QuilA and immune stimulating complexes (ISCOMS).

In order that the invention may be readily understood and put into practical effect, particular preferred embodiments will now be described by way of the following non-limiting examples.

EXAMPLES

EXAMPLE 1

Patients

This prospective study investigated couples participating in ART procedures at The Wesley IVF Service, The Wesley Hospital, Queensland, Australia between September 1999 and May 2001. All consenting couples presenting for a fully stimulated ovarian treatment cycle or a minimal stimulated cycle were enrolled in the project. Stimulation and growth of ovarian follicles in a fully stimulated cycle was achieved with subcutaneous injections of FSH (follicle stimulating hormone) (Puregeon, Organon, Sydney, Australia or Gonalf, Serono Laboratories, Sydney Australia) and nasal application of GnRH analogues to suppress ovulation (Leucrin, Abbotts, Sydney , Australia or Synarel, Monsarto, Sydney, Australia). The growth of the ovarian follicles was monitored by vaginal ultrasound scans every second or third day. Finally the administration of 10,000 U hCG (Profasi, Serono Laboratories, Sydney, Australia) stimulated the maturation and release of the ovarian follicles. Oocytes were retrieved transvaginally 36 hours after the HCG injection under ultrasound guidance. Prior to oocyte collection two endocervical swabs were collected during a speculum examination for subsequent ureaplasma culture. One swab was inoculated directly into 10B Broth (Shepard and Lunceford, 1978) and the second was placed in Stuart's transport media. All clinical samples were frozen at –80° C until processing for culture and PCR.

EXAMPLE 2

Endocervical swabs

Female partner undergoes a stimulated cycle to trigger ovulation of increased numbers of oocytes. Prior to oocyte collection two endocervical swabs were collected during a speculum examination for subsequent ureaplasma culture. One swab was inoculated directly into 10B Broth (Shepard, 1978) and the second was placed in Stuart's transport media. All clinical samples were frozen at –80° C until processing for culture and PCR.

EXAMPLE 3

Semen sample preparation

Semen samples were collected on the day of oocyte retrieval. The semen was collected by masturbation into a sterile container after three days of abstinence. Samples were collected at the IVF laboratory or delivered to the laboratory within 20 minutes of collection. Prior to washing 100 μL of semen was inoculated into 1.8 mL of 10 B broth for ureaplasma culture. The semen was then washed in a PureSperm™ (Genartech, Thornleigh, NSW, Australia) gradient (Mortimor, 1994) consisting of 1 mL 95% PureSperm solution [prepared using culture medium consisting of modified human tubal fluid (HTF) (Irvine Scientific, Santa Ana, CA) supplemented with either 4 mg/mL Albumex or 10% prepared autologous patients' serum]; a 1 mL overlay of 47.5% PureSperm; and an overlay of 1 mL of raw semen. The gradient was centrifuged (Beckman, Allegra-6) for 15 mins at 1000 rpm (300xg) . The pellet was subsequently washed twice in 2-3 mL of culture medium and centrifuged for 10 minutes at 1000 rpm (300 x g). The washed spermatozoa was resuspended in culture medium to a final concentration of <25 million/mL for microdrop (IVF) insemination or fertilisation by ICSI. 200 μL of the washed semen was also inoculated into 1.8 mL of 10B broth for ureaplasma culture. For some patients the spermatozoa were collected by testicular aspiration or alternatively sections of the seminiferous tubules were removed and the sperm was extracted from this tissue (Allan, 1997). This spermatozoa was washed in 80% PureSperm™ prior to ICSI fertilisation (Allan, 1997). Testicular sperm remaining after the completion of the ART procedure was inoculated into 10B broth. All 10 B broth specimens were frozen at –80 ° C until processing.

EXAMPLE 4

Testicular spermatozoa

If desired, the ureaplasmas of the invention can be detected in testicular spermatozoa, which can be collected by testicular aspiration or alternatively sections of the seminiferous tubules can be removed and the sperm was extracted from this tissue (Allan, 1997). These spermatozoa are then rinsed in modified HTF and centrifuged for 15 mins at 1500 rpm (400 x g). The pellet is resuspended in 1 mL of patient Flush medium, gently pipetted to disrupt the tissue, layered over 0.5 mL of 80% PureSperm™ then centrifuged

for 10 mins at 1500 rpm. The pellet is resuspended in flush fluid and motile sperm (twitching) were then selected for ICSI fertilisation (Allan, 1997). Testicular sperm remaining after the completion of the ART procedure is then inoculated into 10B broth. All 10 B broth specimens were frozen at –80° C until processing.

EXAMPLE 5

Swim-up method of sperm preparation.

This preparation protocol is used for semen samples respond poorly to PureSperm™ preparation, or have severe clumping that is exacerbated by PureSperm™ preparation (Osborn)

Sterile 5 mL test tubes are wetted with HTF /4mg/mL albumex™ and then 0.5 mL of semen is placed in each tube. Then 0.5 mL of HTF/albumex™ solution is carefully layered over the top of the semen (a clean interface should be created). The tubes are capped loosely and placed in the incubator for 40 mins and during this time motile sperm should swim up into the HTF/albumex™ solution. If adequate numbers of sperm are present in this layer then this is the final preparation for IVF or ICSI fertilisation. However if the sperm count is low then the HTF/albumex™ layer from all tubes should be combined and centrifuged for 10 mins at 1000 rpm (300 x g). The supernatant is then removed and the pellet resuspended in 1-2 mL of culture medium..

EXAMPLE 6

Ureaplasma culture

All clinical samples (in 10B broth) were serially diluted (three serial 10-fold dilutions) in 10B broth. Ureaplasma growth was detected by an alkaline shift and subsequent colour change in the media. Two aliquots (0.5 mL) of each original specimen were stored at –80° C. All positive semen samples were subsequently quantitated by re-culturing in eight serial 10-fold dilutions of 10B broth. Broths were cultured aerobically, 37° C for 1 week. Ureaplasma positive broth cultures were stored at –80° C.

EXAMPLE 7

DNA Extraction

Stored clinical samples (10B broth) of ureaplasmas were thawed and 500 µL of the sample was centrifuged at 15,900 x g (Beckman Microfuge E) for 20 min at 4° C. The pellet was resuspended in 60 µL of Solution A (10 mM Tris HCl pH 8.3, 100 mM KCl, 2.5 mM $MgCl_2$) and 60 µL Solution B (10 mM Tris HCl pH 8.3, 2.5 mM $MgCl_2$, 1% Tween 20, 1% Triton X-100) with 120 µg/mL of proteinase K. The suspension was incubated for 1 hour at 60° C, then 10 min at 94° C, then cooled and 5- 20 µL of each sample was used for *mba* gene PCR or stored at -20° C until PCR analysis (Blanchard, A., Gautier, M. and Mayau,V., 1991).

EXAMPLE 8

PCR detection

All cultured samples were tested by PCR assays for ureaplasmas and *M. hominis*. The DNA was extracted using previously described methods (Knox and Timms, 1998). Ureaplasmas were detected and speciated using the *mba* primer pair UM-1 (UMS-125 and UMA226) (Teng *et al.* 1993) and previously described PCR conditions (Knox, *et al.*, 1998). All washed semen samples were further tested using a nested PCR reaction: outer primers UMS-125 and UMA 226; and inner primers UMS-9 (5' ATTTTTTATATTAGGAG 3') and UMA163 (5' TTCAATGTCGTAAAC 3'). All clinical samples were also tested by PCR for *M. hominis* using a 16S rRNA assay (Blanchard, 1993).

All ureaplasma isolates were further subtyped using *mba* PCR assays. *U. parvum* isolates were typed by four separate reactions using the primer pairs for *mba* (serovar) 1 (UMS-83 and UMA-41), *mba* (serovar) 3 (serovar 3 UMS-81 and UMA144), and *mba* (serovar) 6 (UMS-53 and UMA122) (Knox and Timms, 1998). *mba* (serovar) 14 was typed using primers serovar 14 UMS –81 (5' AGAAATTATGTAAGATTAAT 3') and UMA144.

All *U. urealyticum* isolates were subtyped into five *mba* types by primers designed from a sequence alignment of the GenBank *mba* sequences of the ATCC strains

of *U. urealyticum* serovars 2, 4, 5, 7-13. These five separate reactions used the upstream primer UMS-7 (5' ATTTCATATTTAGTTTATTAGGSGATCG 3') in combination with UMA 426 (5' TTCCTGGTTGTGTTTCAAAACCTATA 3') for *mba* type 2, 5, 8, 10 (serovars 2, 5, 8 and 10); UMA 429 (5' TGCCTGGTTGTGTTTCG AAACTCC 3') for *mba* type 4, 2 ,13 (serovars 4, 12 and 13); UMA 440 (5' CGTTGGTTCTGGTGTATGAGTTGC 3') for *mba* 7,11 (serovars 7 and 11); UMA 442 (5' GTTCTGGAGTTGGTGTAGGCGC 3') for *mba* 9 (serovar 9); or UMA 461 (5' TTGAACCACTTCCTGGTTGTGTAG 3') for *mba* 10 (serovar 10).

Separate PCRs were performed for each primer pair. The reaction mixture (50 μL) contained 1 U of *Taq* (Boehringer GmbH, Mannheim, Germany), 10x PCR buffer (100 mM Tris-HCl, 15 mM $MgCl_2$, 500 mM KCl, pH 8.3, Boehringer), 200 μM each deoxynucleotide triphosphate (Boehringer), water, 20 pmol of each primer and DNA template (5 to10 μL of prepared sample or 1 μL of extracted DNA). The PCRs were individually optimised and the reaction parameters for each primer pair were critical. The DNA thermal cycler 480 (Perkin-Elmer Cetus) was programmed for: 1 cycle of denaturation, 94° C for 15 min followed by 35 cycles (serovars 3, 6, 14) or 40 cycles (serovar 1) consisting of denaturation at 94° C for 1 min, annealing for 1 min (serovar 1- 57° C; serovar 3 and 14- 52° C; serovar 6-56° C; serovars 2, 4, 5 7-13 – 65° C), and extension at 72° C for 1 min. This was followed by a final cycle of 72° C for 10 min. PCR products were separated by electrophoresis in a 2% agarose gel and visualised by ethidium bromide staining. Reference serovars 1, 3, 6, 8 and 14 and ATCC strains for serovars 2, 4, 5, 7, 9-13 were used as controls.

EXAMPLE 9

Prevalence of ureaplasma subtypes in clinical samples

Clinical samples for ureaplasma culture and PCR detection were collected from 179 couples participating in ART treatment cycles. Ureaplasmas were detected in: 22.9% (41/179) of semen samples; 10.6% (19/179) of washed semen samples; and 44.7% (80/179) of endocervical samples. All clinical ureaplasma isolates were subtyped using PCR assays (TABLE I). *U. parvum* isolates were detected more frequently in both men (80.5% of isolates) and women (93% of isolates). However there was a higher incidence of *U. urealyticum* in men (19.5% of isolates) than in women (7 % of isolates). Mixed serovars

were detected in 5/179 semen samples (6.7%) and 11/179 endocervical samples (6%). In 9/179 (5%) couples the male and female partners were found to be infected/colonised with different ureaplasma subtypes. *U. parvum* serovar 3 was isolated most frequently from the endocervix of female partners (43%), and from male semen (35%). However, *U. parvum* serovar 6 is the most prevalent subtype in male washed semen (37%). Serovar 6, has a stronger *in vivo* binding affinity for spermatozoa than serovars 1 and 3, and was not removed by washing in a pure sperm 95/47.5 gradient procedure prior to an ART treatment in 58% of men colonised/infected with serovar 6. By contrast 78% of serovar 1 and 69% of serovar 3 clinical isolates were removed from the spermatozoa by this standard washing procedure. *U. urealyticum* subtype *mba* 2, 5, 8 and 10 was not removed by washing in 2 of the 6 typed isolates. Typing of the remaining *U. urealyticum* isolates may yet reveal further adherent subtypes.

EXAMPLE 10

Comparison of fertilisation rates of ureaplasma washed sperm positive couples and ureaplasma washed sperm negative couples

The fertilisation rates for ureaplasma washed semen positive couples and ureaplasma washed semen negative couples (three separate ureaplasma classifications: sperm and endocervical positive; sperm negative endocervical positive; sperm and endocervical negative) were compared (TABLE II). Overall a higher rate of oocyte fertilisation was achieved using the ICSI procedure (average 72.8%) than by conventional IVF (average 69.5%).. However, these data show a reduced IVF fertilisation rate for ureaplasma washed sperm positive couples (64 %) compared to the three classifications of washed sperm negative couples (74%, 75%, and 68% respectively).

EXAMPLE 11

Comparison of the total number of pregnancies achieved by fresh and frozen embryo transfer after fertilisation by either IVF or ICSI techniques

Pregnancy outcome data (TABLE III) for these patients shows no significant difference in the viable pregnancy rate in couples with ureaplasma positive washed semen (13 %) compared to those couples with ureaplasma negative washed semen (26 %, 13% and 15% respectively). However if we consider the pregnancy outcomes for IVF and ICSI

pregnancies separately (TABLE IV and TABLE V) a greatly reduced viable pregnancy rate is observed for ureaplasma washed sperm positive ICSI couples (6.7%) but not for ureaplasma positive washed sperm IVF couples (20%).

There was an increased miscarriage rate in couples with ureaplasma positive washed sperm (56%) compared to couples with ureaplasma negative washed sperm (11%, 25% and 35% respectively). An increased miscarriage rate is demonstrated in both IVF (50%) and ICSI (66%) ureaplasma washed sperm positive couples when compared to the three separate ureaplasma classifications: sperm and endocervical positive (0%, 17% respectively); sperm negative endocervical positive (29%, 20% respectively);and sperm and endocervical negative (35%, 35% respectively) (TABLE IV and TABLE V).

Conclusions

Not wishing to be bound by any one particular theory or mode of operation, the inventors believe that the mechanism by which infection of the embryo and the placenta are initiated is central to elucidating the pathogenesis of the ureaplasmas in adverse pregnancy outcome. In this light, it is proposed that ureaplasmal infection of the embryo and the placenta occurs as a result of fertilisation of an oocyte by spermatozoa with adherent ureaplasmas and the results from this current study add further support to this hypothesis.

Electron microscopy has shown that ureaplasmas attach firmly to the midpieces and post-acrosomal regions of spermatozoa (Xu, *et al.*, 1997). In this study, the inventors have demonstrated (by culture and PCR assays) that ureaplasmas were not removed from 46% of infected/colonised semen samples by washing in a Pure sperm 95/47.5 gradient and this confirms there is a risk that the embryo may become infected during ART fertilisation procedures. Significantly the present inventors have also shown that *U. parvum* serovar 6 has a stronger *in vivo* binding affinity for spermatozoa and were not removed from semen in 58% of those infected/colonised with this serovar. By contrast *U. parvum* serovar 1 was the least adherent to spermatozoa and was removed from 78% of ureaplasma positive semen samples by the washing procedure. This current study has also confirmed that ureaplasma positive semen does not affect the fertilisation rate using either ICSI of IVF techniques. However the inventors have shown a slighty reduced IVF fertilisation rate (but not the ICSI rate) in couples with ureaplasma positive washed semen (64%) compared to

couples with ureaplasma negative washed semen (average 70%) (TABLE II). Previously Busolo and Zanchetta (1984) (Busolo and Zanchetta,1984) demonstrated reduced oocyte penetration rates by spermatozoa pre-incubated with ureaplasmas (serovars 1-8). The greatest reductions in oocyte penetration were observed for spermatozoa pre-incubated with serovar 6. The least reduction in penetration was observed for serovar 1 – the serovar, which was less adherent to spermatozoa and removed more frequently by washing in this current study. These results suggest that the binding affinity of the different ureaplasma serovars to spermatozoa could be an important determinant affecting oocyte penetration and fertilisation.

The present investigation has also demonstrated a reduced viable pregnancy rate in ureaplasma positive washed semen ICSI couples and a an increased miscarriage rate in all couples with ureaplasma positive washed semen, but not in couples with ureaplasma positive semen or in couples with ureaplasma positive endocervical samples (TABLE III, IV and V). During ICSI fertilisation techniques, ureaplasmas adherent to spermatozoa in washed semen samples are injected into and infect the oocyte. In ICSI patients there is only one continuing viable pregnancies (1/15 embryo transfers) and the results herein reveal a 66%(2/3 pregnancies) miscarriage rate in ureaplasma washed sperm positive couples. There is evidence that the oocyte also becomes infected during IVF procedures. In ureaplasma washed semen positive IVF patients there is a 20% viable pregnancy rate and a 50% miscarriage rate. Whilst these rates are still different to ureaplasma negative washed semen couples, it appears that natural selective processes may in some instances facilitate fertilisation by spermatozoa without adherent ureaplasmas and thus prevent infection at conception. Whilst *U. parvum* serovar 6 was isolated from the washed semen of all ICSI couples (ureaplasma washed semen positive couples) who miscarried, *U. parvum* serovars 3, and 6 and *U. urealyticum* were isolated from the washed semen of IVF couples who miscarried.

Whilst the sample size of this study is still too small to demonstrate statistical significance, the above results strongly support the hypothesis that ureaplasma adherence to spermatozoa is a mechanism of pathogenicity facilitating infection of the embryo at conception. The teachings described herein have implications for the future treatment of not only ART patients but also couples with a recurrent history of preterm delivery due to ureaplasma chorioamnionitis. Screening of couples prior to an ART treatment cycles will

identify couples with adherent ureaplasmas in washed semen samples. Antibiotic treatment of both partners just prior to oocyte retrieval could eradicate ureaplasmas and improve ART pregnancy outcomes.

EXAMPLE 12

Identification of adhesin - U. parvum serovar 6

Interactions of whole cell ureaplasmas (previously characterised as adherent and non-adherent, *in vivo* and *in vitro)* with SGG (purified from bull testes, Lingwood *et al.* 1980) and SCG (Sigma) are compared using real-time biomolecular interaction analysis (BIA) in a BIAcore™ 2000, a system that uses surface plasmon resonance (SPR). The SGG (or SCG) is bound to the surface of a sensor chip. Briefly, liposomes of dimyristoylphosphatidylcholine in buffer are seeded (1-5%) with SGG, and are prepared by extrusion through 50 nm pore size filters. The liposomal mixture (the ligand) is allowed to flow through the BIAcore™ 2000 and is bound to L1 sensor chips (BIAcore™ AB, Australia). Whole cell ureaplasmas (analytes) in solution are run over this surface and the interaction is followed in real time by SPR analysis. Low flow rates and sample recovery are used to ensure whole cells are not lysed by these procedures. The advantages of using this system are that: whole cell ureaplasmas can be used; interactions are followed in real time without labelling of either the cells or the immobilised ligand; the relative affinities of different ureaplasma serovars for either ligand can be determined; and the kinetics of the different interactions of different ureaplasma serovars directly compared. Mixtures of clinical ureaplasma serovars can also be analysed to determine if there is preferential binding to the ligands of particular serovars from a mixed population. The preferentially bound serovars will be identified after sample recovery.

Cell lysates and membrane preparations from (clinically defined) strongly-adherent and non-adherent serovars are compared for their ability to interact with SCG in the BIAcore™ 2000. Whole cell ureaplasmas are pre-treated with proteases (papain, trypsin, chymotrypsin) or heat in the presence of reductants (β-mercaptoethanol) to determine if the SGG receptor is proteinaceous. Preparations of the proteinaceous receptor are suitably examined by SDS-PAGE for any obvious differences in protein composition, but, importantly, the recovery option of the BIAcore™ 2000 is used to capture any specifically bound material for similar examination. Recovered material, either from

BIAcore™ or excised from PVDF membrane following Western blot is N-terminally sequenced by Edman degradation, mass spectrometry or both.

In another example, trypsin or chymotrypsin pre-treatment of adherent ureaplasma cells is used to prevent adherence, and subsequently the proteolytic fragments produced by this treatment are examined for their ability to bind to SGG. If necessary, fractionation of these fragments by reverse-phase HPLC over C4 or C8 matrices is used to determine the identity of the fragment(s) specifically involved in such interactions. The fragments with a demonstrated binding ability to bind SGG are be characterised by N-terminal sequencing or mass spectrometry, or both, and compared to commercially available protein databases and the complete sequence of *U. parvum* serovar 3 (Glass *et al.* 2000) for identification.

In yet another example, an adhesin may be identified by comparing surface accessible membrane proteins of adherent (serovar 6) and non-adherent (serovar 1) ureaplasma isolates (first passage clinical isolates). A number of strategies known to persons of skill in the art can be employed in this regard.

The disclosure of every patent, patent application, and publication cited herein is hereby incorporated herein by reference in its entirety.

The citation of any reference herein should not be construed as an admission that such reference is available as "Prior Art" to the instant application.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

DATED this Eighteenth day of May, 2001

QUEENSLAND UNIVERSITY OF TECHNOLOGY

By their Patent Attorneys

DAVIES COLLISON CAVE

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TABLES

TABLE 1

Prevalence of ureaplasma subtypes in clinical samples

Ureaplasma subtypes	*Semen*	*Washed Semen*		*Endocervical swabs*
U. parvum serovar 1	9	2		14
U. parvum serovar 3	16	5		39
U. parvum serovar 6	12	7		31
U. parvum serovar 14	0	0		0
Ureaplasma urealyticum	9	5		6
No. of isolates	46 / 41 samples	19/19samples		91/80 samples

TABLE II

Comparison of fertilisation rates of ureaplasma washed sperm positive couples and ureaplasma washed sperm negative couples

Method of fertilisation	*Ureaplasma Washed Sperm +ve Sperm +ve Endocervical +ve*	*Ureaplasma Washed sperm -ve Sperm +ve Endocervical +ve*	*Ureaplasma Washed sperm -ve Sperm -ve Endocervical +ve*	*Ureaplasma Washed sperm -ve Sperm -ve Endocervical -ve*
ICSI fertilisation rate*	74/104 71%	84/109 77%	159/229 69%	272/367 74%
IVF fertilisation rate†	64/100 64%	85/115 74%	107/143 75%	221/340 65%
Total rate of fertilisation	138/204 68%	169/224 75%	266/ 372 71%	493/707 70%

* no. fertilised oocytes/ no. injected with spermatozoa, † no. fertilised oocytes/ total no. of oocytes retrieved

TABLE III

A comparison of the total number of pregnancies achieved by fresh and frozen embryo transfer after fertilisation by either IVF or ICSI techniques Replace withthe table below

	Ureaplasma Washed sperm +ve Sperm +ve Endocervical +ve ET=11	*Ureaplasma Washed sperm -ve Sperm +ve Endocervical +ve ET=36*	*Ureaplasma Washed sperm -ve Sperm -ve Endocervical +ve ET=59*	*Ureaplasma Washed sperm -ve Sperm -ve Endocervical –ve ET=140*
Clinical preg/ET	4 36%	13 36%	8 14%	34 24%
Viable preg/ET	1 9%	11 31%	7 12%	24 17%
Miscarriages/clin preg	¾ 75%	2/13 15%	0	9/34 26%
Termination				1
Ectopic	0			1
TOTAL	*Ureaplasma Sperm +ve Washed sperm +ve Endocervical +ve ET=30*	*Ureaplasma Sperm +ve Washed sperm -ve Endocervical +ve ET=31*	*Ureaplasma Sperm -ve Washed sperm -ve Endocervical +ve ET=60*	*Ureaplasma Sperm -ve Washed sperm -ve Endocervical –ve ET=138*
Total preg/ET	9 30%	9 29%	12 20%	34 25%
Viable preg/ET	4 13%	8 26%	8 13%	20 15%
Miscarriages/Tot preg	5/9 56%	1/9 11%	3/12 25%	12/34 35%
Termination				1
Ectopic			1	1

TABLE IV

A comparison of the total number of pregnancies achieved by fresh and frozen embryo transfer after fertilisation by ICSI techniques

ICSI TOTAL	*Ureaplasma Sperm +ve Washed sperm +ve Endocervical +ve ET=15*	*Ureaplasma Sperm +ve Washed sperm -ve Endocervical +ve ET=22*	*Ureaplasma Sperm -ve Washed sperm -ve Endocervical +ve ET=30*	*Ureaplasma Sperm -ve Washed sperm -ve Endocervical -ve ET=75*
Total preg/ET	3 20%	6 27%	5 17%	17 23%
Viable preg/ET	1 6.7%	5 23%	3 10%	11 15%
Miscarriages/Tot preg	2/3 66%	1/6 17%	1/5 20%	6/17 35%
Ectopic			1	

TABLE V

A comparison of the total number of pregnancies achieved by fresh and frozen embryo transfer after fertilisation by IVF

IVF TOTAL	Ureaplasma Sperm +ve Washed sperm +ve Endocervical +ve Et=15	Ureaplasma Sperm +ve Washed sperm -ve Endocervical +ve ET=9	Ureaplasma Sperm -ve Washed sperm -ve Endocervical +ve Et=30	Ureaplasma Sperm -ve Washed sperm -ve Endocervical -ve Et=63
Total preg/ET	6 40%	3 33%	7 23%	17 27%
Viable preg/ET	3 20%	3 33%	5 17%	9 14%
Miscarriages/Tot preg	3/6 50%	0 0%	2/7 29%	6/17 35%
Termination				1
Ectopic				1

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